VOLUME XVIII MARCH, 1935 NUMBER 3

JOURNAL OF DAIRY SCIENCE

PUBLISHED BY THE
AMERICAN DAIRY SCIENCE ASSOCIATION

Contents

The relation of the fat content of milk to the passage of the curd from the stomach of the calf. D. L. Espe and C. F. Cannon 161

The mechanical control of the fat content of Swiss cheese. Warren V. Price and Gerald C. Hoppe 181

Dominant digestion without roughage. S. W. Mead and Hudson Gore 193

The use of sodium thiosulphate dilution blanks in determining the germicidal efficiency of chlorine sterilizers. C. C. Pickett and Roy T. Olson 211

A method for the determination of the relative stiffness of cream during the whipping process. W. S. Mortram 217

Nutritional anemia, calcium, phosphorus and nitrogen balance and bone composition of rats fed raw versus pasteurized milk. H. A. Lardy and L. S. Palmer 223

Association announcements 238

THE SCIENCE PRESS PRINTING COMPANY
Lime and Queen Streets, Lancaster, Pa.
Entered as second-class matter at the Post Office at Lancaster, Pa., under the Act of March 3, 1879.

PRECISION PASTEURIZER

flexibility

GIVES YOU MADE-TO-ORDER equipment at "ready-made" prices

Because CP Precision Heaters are made in standard sections, in one or two stands and in two sizes of tubes, we can offer 44 different combinations in our 1935 line.

Capacities range from 4000 to 24000 pounds per hour for temperature differentials (water and milk) of one, four and eight degrees, respectively. One series is especially adapted to high-temperature, short-time, pasteurization.

Corresponding in capacity to our Precision Tank Holder Systems, they make a most flexible system. Either heating or holding capacity can be increased at any time by simply adding another tank to the holder, or changing its timing, and adding another section to the heater.

Thus your investment is safe and protected. Never will you be required to junk expensive equipment because it cannot be enlarged to care for needed additional capacity.

Precision Tank Holder. Simplest and safest of all holders because of Precision Holder Valves.

Precision Heater—single stand—18 tubes. Sizes for every need up to 24,000 lbs. per hour.

Buying pasteurizing equipment is not an everyday affair. Mistakes are expensive and even more expensive to rectify. They can be avoided if you'll call on CP to fit equipment to your needs.

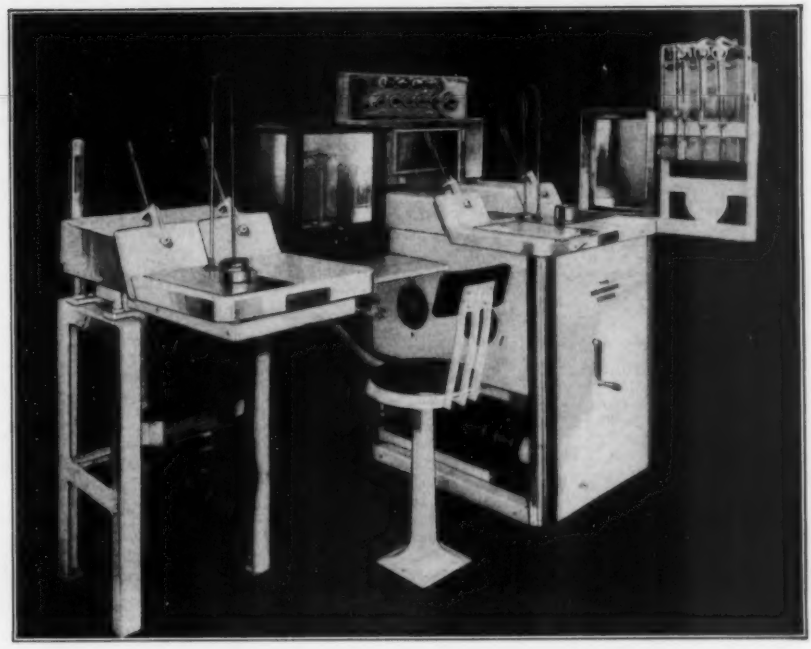
Your inquiry is invited

Creamery Package

THE CREAMERY PACKAGE MFG. COMPANY, 2900 West Washington Blvd., Chicago, Ill.

Entered as second-class matter April 19, 1934, at the Post Office at Lancaster, Pa. Under the Act of March 3, 1879.

Copyrighted, 1935, by the Atlantic Dairy Science Association.



SCIENTIFIC ACCURACY

in the testing of milk and milk products is even more necessary in research and laboratory work than it is in the daily operation of a milk plant.

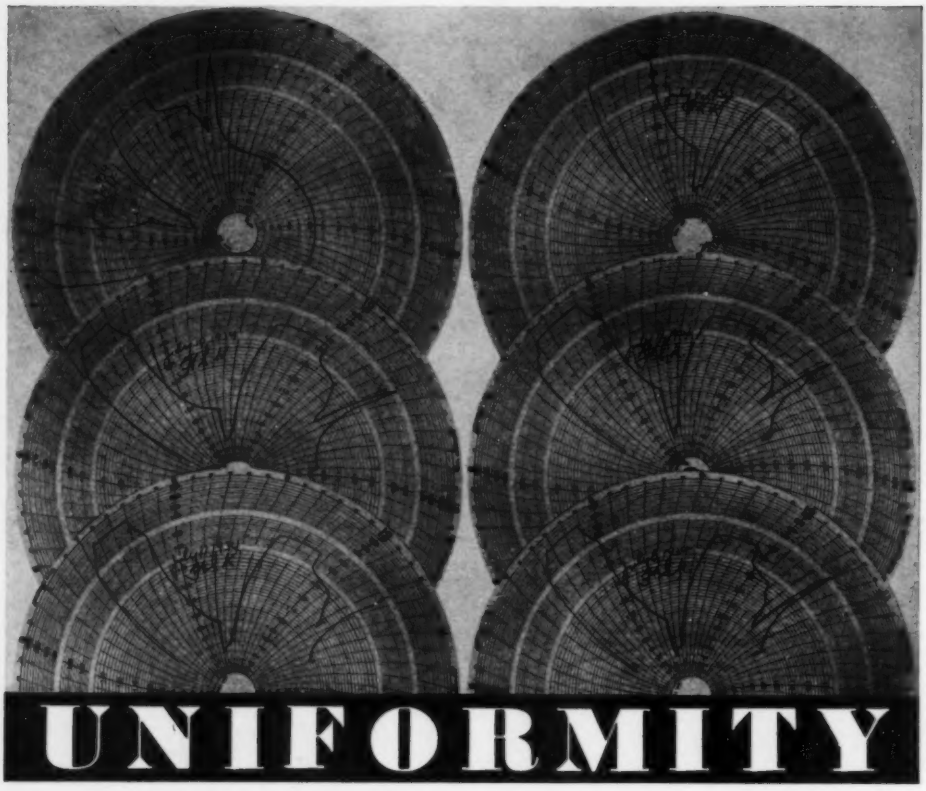
The Mojonnier Tester for determining the butter fat and total solids content of milk provides this great accuracy and at the same time permits tests to be made rapidly, the average time per test being less than 30 minutes. The Mojonnier Tester is the only apparatus on the market which provides a means of making butterfat and total solids tests on the same sample simultaneously. For colleges, agricultural experiment stations and other laboratories where a large number of tests are needed daily, the Model D shown above provides ample capacity. Another model having a smaller capacity is also available.

Full particulars will be gladly sent upon request.

MOJONNIER BROS. CO.
4601 W. Ohio St., Chicago, Ill.

Mojonnier MILK TESTER

Your advertisement is being read in every State and in 25 Foreign Countries



UNIFORMITY

Pasteurization of the sort that is most successful . . most thorough . . lowest in cost . . with deepest cream layer and finest flavor . . is pasteurization which features positive uniform heating and holding with minimum attention from the operator. In other words . . pasteurization in CHERRY-BURRELL SERIES "CC" SPRAY PASTEURIZERS with SENTINEL CONTROLS.

Typical of such uniformity are the recording thermometer charts reproduced above which were sent in to us by Mr. Elmer Kleiser of the Buffalo Valley Dairy of Milton, Pa. Mr. Kleiser says that their 200-gallon series "CC" Spray Pasteurizer with its Sentinel Control is so accurate, positive and dependable that they *"have never watched the milk being pasteurized at any time"* and they have never needed to call for a service man. Ask for bulletins about this remarkable machine. Enjoy the same trouble-free, automatic, efficient pasteurization. The Series "CC" Spray Pasteurizer, remember, is made in a wide range of capacities. CHERRY-BURRELL CORPORATION, 427 W. RANDOLPH ST., CHICAGO, ILL.

Your advertisement is being read in every State and in 25 Foreign Countries

JOURNAL OF DAIRY SCIENCE

VOLUME XVIII MARCH, 1935 NUMBER 3

THE RELATION OF THE FAT CONTENT OF MILK TO THE PASSAGE OF THE MILK CURD FROM THE STOMACH OF THE CALF*

D. L. ESPE AND C. Y. CANNON

Iowa State College, Ames, Iowa

When the calf has reached the age of three weeks or more, it is the practice of most dairymen to replace the whole milk with skim milk, supplemented by grain and hay. The removal of the fat from the milk narrows the nutritive ratio of the milk from 1:3.9 to 1:1.5. The addition of grain and roughage tends to widen this ratio again. Though the calf's stomach is especially designed to handle large amounts of fiber, there is still some question as to the age at which it is most practical to begin replacing a fiber-free energy food, like fat, with large amounts of grain and roughage.

In addition to the physiological effect of bulk the omission of fat may be of importance in other ways not generally recognized. For instance, the calf is very susceptible to bowel disturbances during the first few weeks of its life. Favorite remedies at this time have been to dilute the milk with lime water, add barley water, or, boil the milk. All of these modifications of milk are designed to make it more digestible in the sense that a more friable, more easily liquefied curd is formed in the stomach. Other nutritive changes may of course accompany these modifications. On the other hand it is generally recognized (3), (4), (7), that fat tends to inhibit the rate of evacuation of the stomach. Alley, MacKenzie and Webster (1) are of the opinion that fat diminishes the peptic power of the gastric juice. The question immediately arises, does the amount of fat commonly found in milk play a favorable or unfavorable part during these periods of indisposition? Is it desirable or undesirable to speed up the rate of evacuation of the stomach at this time and what part does the fat in whole milk play in controlling the stomach processes?

The nutritive value of the fat is unquestioned. Not only does fat serve as a source of energy and as a carrier of the fat soluble vitamins but possibly as the source of certain fatty acids essential to life. However, the

Received for publication October 22, 1934.

* Journal Paper No. J193 of the Iowa Agricultural Experiment Station. Ames, Iowa. Project No. 47.

economy of whole milk as a food source makes a knowledge of the body reserves of these elements important.

EXPERIMENTAL

Two trials in consecutive years were run in obtaining the data to be reported. In both trials, calves with rumen fistulas were used. The fistulas were usually made when the calves were about two weks old. The test meal was placed in a liter bottle and allowed to flow directly into the abomasum through a one-quarter inch rubber tube. By running the tube through the fistula directly into the stomach the effects of dilution by saliva or fluid in the rumen was avoided. No grain or hay was fed while the trials were in progress. At varied intervals, however, the muzzles were removed and the calves allowed to eat *ad libitum*. Before a trial was started the rumen and reticulum were washed out. Sufficient time was then allowed to elapse until no food could be found in the omasum or abomasum. A mineral mixture of salt, steamed bone meal, iron oxide, copper sulfate and potassium iodide was fed, supplemented by cod liver oil.

The time required for liquefaction and evacuation of the stomach was determined by palpating the curd mass through the walls of the rumen and abomasum. In calves, the abdomen lies almost directly beneath the rumen when the latter is empty. As the walls of both stomachs are relatively soft and elastic, any object the size of a pea or larger can be readily located in the abomasum by palpating the floor of the rumen. In fact the authors feel that the exact time at which all food leaves the stomach can be more readily determined in this way than by either Röentgen ray photographs or by acidity curves. The use of acidity curves to supplement the palpation method would of course, be desirable. However, no method probably disturbs the normal physiological processes less than palpation if the effect of barium sulfate in the meal is considered in the taking of Röentgen ray photographs. In fact, after some experience the hand can be inserted into the rumen without the calf being scarcely more than conscious of the act.

For the first few trials the abomasum was palpated every hour after feeding. Near the end of the trial the stomach was palpated every fifteen to twenty minutes to determine the exact end point. When the first few trials were completed and the length of the digestive period for the respective calves determined, then only the palpations at the end of the period were required.

Digestion was considered sufficiently complete when only small amounts of curd (5 to 10 grams) could be located by palpating the abomasum. As soon as this stage was reached the fingers were inserted into the abomasum and the remaining curd removed and weighed. These curds served as a check on the end point of digestion.

In the first series of trials (5) the milk from two Holstein cows was

mixed and used. All milk was separated, standardized to the desired fat content and then viscolized to prevent the fat from separating out. The skim milk was also viscolized. The skim milk trials were conducted first, followed by those with three and six per cent milk respectively.

In the second series of trials an attempt was made to avoid any effect of advancing age by alternating the skim milk trials with those comparing three per cent and six per cent milk. The average time for the evacuation of the stomach with each type of test meal was then computed for each calf. The calves were not fed for 24 hours previous to being given the test meal. Eight hours before the test meal was given the abomasum was examined for any food present and all curds removed manually. Never more than two trials were carried out in any one week in order that the calves might regain any lost weight as a result of the previous trial. The milk used throughout the second series of trials came from different cows from those used in the first series but the same milk was used in each trial. However, the curd tension of this milk was very similar to that used the previous year. In these latter trials whole milk was used instead of milk standardized to three per cent fat. The milk actually tested about 2.8 per cent. The milk standardized for the high fat trials actually tested 5.8 per cent.

Two other slight differences between the two series should be mentioned. In the first series, no attempt was made to control the rate of flow of the milk into the stomach nor was the temperature of the milk closely controlled except to be sure that it was warm. In the second series of trials the rate of flow of the milk was adjusted so that approximately two minutes were required for the test meal, one liter, to flow into the stomach. This was done in an attempt to simulate conditions occurring when the calf sucks the cow. The temperature of the milk was also closely controlled between 35° and 37° C.

RESULTS

From table 1 it will be seen that in each case one liter of skim milk required longer to leave the stomach than one liter of milk containing three per cent fat. Also, one liter of three per cent milk required longer to leave the stomach than the same amount of milk containing six per cent fat. The difference in time between the trials with skim milk and those with milk containing three per cent fat is greater, however, than between the trials with milk containing three and six per cent fat.

In table 2 the same general trend is observed though the results are less uniform. No figures are given for the amount of curd removed as an arbitrary value was chosen by which the time for complete evacuation of the stomach was calculated. Curds weighing five grams or less were assumed to "digest" at the rate of five grams per hour. Curds weighing six to ten grams were assumed to "digest" at the rate of six grams per hour. No dif-

TABLE 1

The relation of the fat content of milk to the passage of the milk curd from the stomach of the calf

Series 1

KIND OF MILK	TRIAL NO.	CALF 47J		CALF 47K		CALF 47L		CALF 47M		AVERAGE OF ALL TRIALS
		Evac. time in min.*	Wt. of curd in grams**	Evac. time in min.	Wt. of curd in grams	Evac. time in min.	Wt. of curd in grams	Evac. time in min.	Wt. of curd in grams	
	1	340	2.6	345	2.3	315	0.3	425	4.5	Digestion
	2	335	16.1	320	8.3	300	4.9	345	2.9	period
Skim milk	3	310	7.4	320	1.3	300	1.8	355	2.6	325 min.
	4	310	6.2	305	6.5	270	6.1			
	5	305	3.1	305	0.4					
	6	305	1.4	285	3.6					Weight of curd
Average		317	6.1	313	3.7	296	3.3	375	3.3	3.9 grams
	1	275	5.7	290	6.1	350	1.9	315	9.5	Digestion
	2	265	1.1	290	0.6	265	2.7			period
3 per cent milk	3	255	2.4	270	3.2					281 min.
	4	235	1.4	260	5.0	245	0.3	310	1.6	
	5	235	1.1	240	2.5	215	2.3	305	3.6	
	6	225	3.4	220	1.9					Weight of curd
Average		248	2.5	262	3.2	269	1.8	310	4.9	3.3 grams
	1	275	0.9	285	3.9	285	1.3	260	2.5	Digestion
	2	265	1.4	285	2.1	265	0.3	245	3.5	period
6 per cent milk	3	230	2.1	250	3.6	245	2.4	240	1.3	239 min.
	4	225	1.9	235	3.2	230	2.1			
	5	220	1.1	225	0.5					
	6	215	3.5	215	1.4					Weight of curd
Average		238	1.8	249	2.5	256	1.5	248	2.4	2.0 grams

* Length of time from the ingestion of the milk to the removal of any unliquefied curd.

** Dry weight of curd still remaining in the stomach at the end of the test period.

TABLE 2

The relation of the fat content of the milk to the passage of the milk curd from the stomach of the calf

Series 2

CALF NO.	NO. OF TRIALS	KIND OF TREATMENT	TOTAL TIME FOR LIQUEFACTION OF CURD IN STOMACH
47–R	9	Skim milk	16 hrs. 20 min.
"	14	3 per cent milk	12 " 22 "
"	4	6 per cent milk	10 " 39 "
47–X	7	Skim milk	16 hrs. 26 min.
"	9	3 per cent milk	12 " 57 "
"	4	6 per cent milk	12 " 3 "
47–A–3	4	Skim milk	19 hrs. 13 min.
"	5	3 per cent milk	17 " 21 "
"	6	6 per cent milk	15 " 45 "
47–A–4	6	Skim milk	17 hrs. 41 min.
"	6	3 per cent milk	15 " 35 "
"	5	6 per cent milk	13 " 15 "
	Total Number Trials		
Average of 4 calves	26	Skim milk	17 hrs. 25 min.
	34	3 per cent milk	14 " 34 "
	19	6 per cent milk	12 " 56 "

ference was made between curds from skim milk, milk containing three or six per cent fat.

DISCUSSION

In this study no measure was made of the stage of proteolysis reached by the milk curd but simply determinations of the rates at which the protein curds were liquefied and left the stomach. Several physiological disturbances, however, may have influenced liquefaction. Even the same stomach rarely digested aliquot samples of milk in the same length of time, while the variation in digestion time between the different calves was even greater.

Though there is some indication that the curd formed from skim milk leaves the stomach more slowly than when either three or six per cent fat has been added, the difference is not highly significant due to the marked variation in evacuation time between trials with the same kind of milk. Changes in barn temperature, appetite and the changing state of health of the animals made it impossible to reduce these daily fluctuations to the point desired. In fact, the length of the digestive period was a good index to the general physical condition of the calves.

No definite explanation can be given to show why the calves in one series always "digested" the test meals more rapidly than those of the other series. The viscolization of the milk may have been responsible for the difference.

Quigley, Zettleman and Ivy (6) state that the inhibitory action of fats on gastric secretion and gastric motility is due to a humoral effect set up by the fats while in contact with the mucosa of the small intestine. Alley, MacKenzie and Webster (1) are of the same opinion except that they lay more stress upon the nervous phase of gastric secretion. Apparently the calf does not have such a mechanism or else the percentage of fat in the milk is not sufficient to give a pronounced inhibitory effect. The last explanation would seem much more logical. In fact, Boggess, Bessie and Ivy (3) found that limited amounts of fat did not inhibit the passage of food from the stomach. Bergeim and coworkers (2) found that skim milk required longer to liquefy and escape from the stomach than did milk with four per cent fat. In our own experiments the curd which was formed from skim milk became semi-opalescent in color and tenacious, almost like rubber, in texture while the curd from milk containing six per cent fat was very friable, like cottage cheese in consistency. The curd from milk containing three to six per cent of fat produces a much softer curd in the stomach of the calf than fat-free milk while this amount of fat is sufficiently low to not inhibit the normal functioning of the stomach. It would appear, therefore, that the action of the fat is not antagonistic to the commonly accepted methods of making the milk more digestible, such as diluting with lime water or barley water, or boiling. Though the abomasum of the calf being fed milk is rarely if ever empty, the normal amount of fat present in the milk will tend to produce a softer more friable curd which usually leaves the stomach more quickly than curd formed from skim milk.

SUMMARY

Although whole milk has always been considered preferable for calf feeding to separator skimmed milk from a growth promoting standpoint, the cost of feeding whole milk makes it desirable to change to skim milk feeding at the earliest possible age. In this paper the physiological effect of the fat on the rate of evacuation of the stomach is discussed. Curd from milk containing up to six per cent fat tends to leave the stomach more rapidly than skimmed mild due to the difference in texture of the curd formed. Milk containing this amount of fat does not apparently inhibit gastric secretion or gastric motility. Fat, in addition to being a valuable source of food, would appear to aid digestion when incorporated in the milk in limited amounts.

REFERENCES

(1) Alley, Armine, MacKenzie, D. W., and Webster, D. R. Dissociation of the functional properties of the gastric glands under the influence of fat. Amer. Jour. of Dig. Dis. and Nutr. 1: 333–336. 1934.

(2) Bergeim, Olaf, Evvard, J. M., Rehfuss, M. E., and Hawks, P. B. The gastric

response to foods. A fractional study of the coagulation of milk in the human stomach. Amer. Jour. of Physiol. **48**: 411–18. 1919.

(3) Boggess, Bessie, and A. C. Ivy. The digestibility of potatoes as influenced by methods of preparation. Jour. Home Ec. **19**: 496. 1927.

(4) Cannon, W. B. The passage of different foodstuffs from the stomach and through the small intestine. Amer. Jour. of Physiol. **12**: 387–418. 1904.

(5) Hargrave, R. The relation of the fat content of milk to the passage of milk curd from the stomach of the calf. Unpublished Thesis, Library, Iowa State College. 1933.

(6) Quigley, J. P., Zettelman, H. J., and Ivy, A. C. Analysis of the factors involved in gastric motor inhibition by fats. Amer. Jour. of Physiol. **108**: 643–651. 1934.

(7) Tobler, L., and Bogen, H. Monatsschrift für Kinderheilkunde, **7**: 12. 1908–1909.

THE MECHANICAL CONTROL OF THE FAT CONTENT OF SWISS CHEESE

WALTER V. PRICE AND GERALD C. NORTH

Department of Dairy Industry, College of Agriculture, Madison, Wis.

The eye formation in Swiss cheese is one of the most important factors in determining its market grade. Inspectors commonly establish this grade by simply drawing and examining two or three plugs from a wheel of Swiss without either smelling or tasting the samples. Dealers justify this procedure by stating that the trade is chiefly interested in the eye formation and slicing properties and that other characteristics are of so little significance that they may be practically neglected in all except unusual circumstances. Connoisseurs of cheese, who are aware of this condition, believe that subordination of flavor and body to eye formation and slicing properties will eventually injure the sale of Swiss cheese.

Within a radius of 50 miles from Monroe, Wisconsin, are located nearly 200 Swiss cheese factories. During the season of greatest production in 1933, a study was made in this area to determine, under factory conditions, the relation of the mechanical control of the fat content of the cheese to the composition and commercial quality of the finished product.

The ideal formation of eyes in Swiss cheese depends upon the interrelated effects of biological and mechanical control of the curdmaking and ripening processes. Biological control is attained by using cultures of desirable organisms (1, 2, 3, 4) and directing their activities by regulating those factors which influence their life processes. Mechanical control is established by clarification and removal of fat from the milk (6) and by those manipulations in the curdmaking and ripening operations which assist in determining the composition of the finished product. The relation of these two general methods of controlling formation of eyes in Swiss cheese is such that, in factory practice, it may be impossible to state whether these manipulations are essential because they influence most the biological changes, the physical properties or the chemical composition of the cheese.

METHODS

Selection of factories.—Twenty factories were selected for intensive study. They ranged in size from one to ten kettles and were located in the area approximately according to the intensity of production. Only those factories were selected in which the cheesemakers were recognized leaders in their art.

Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

Received for publication September 17, 1934.

Samples.—Each of these factories was visited at intervals of approximately one month. The visits began on June 20th and ended October 31st, 1933. Samples of milk, whey, curd, and cheese were taken at certain points in the making process that are designated in table 2.

Methods of analysis.—The Babcock test was used for determining the fat content of the various samples. Casein was measured by the Walker Formol Titration. Dry matter was found by drying the samples of curd and cheese at 100° C. until a constant weight was attained.

Methods of grading.—The grades of cheese were determined at approximately 9 weeks of age by representatives of the dealers who purchased them. Makers were always present when the buyer examined the cheese and could protest any unfair grade. Such disagreements arose very infrequently. There was no supervision of grading by any State official during the period of the study. The characteristics of the four market grades, which were recognized by the dealers, are classified in table 1. Grade I designates the best quality while a grade of IV indicates the most undesirable cheese and is commonly called a "grinder."

TABLE 1

Description of the market grades of cheese made between June 20, 1933, and October 31, 1933

GRADE	I	II	III	IV
Eyes	Well Developed 1 to 5 per trier	Well Developed About 5 per trier	Overset	Blind
Texture	No Glass nor Pinholes	No Glass nor Pinholes	Glass No Pinholes	Pinholes
Body	Firm and Meaty	Fairly Firm and Meaty	Fairly Firm and Meaty	Fails to Meet Requirements for other Grades
Flavor	Clean, Desirable, Characteristic of Age	Clean, May Lack Flavor	May Lack Flavor, Not Objectionable	Fails to Meet Requirements for other Grades
Finish	Well-shaped, Clean, Sound Rind	Well-Shaped, Clean, Sound Rind	Clean, Well-shaped, Sound Rind, Slightly Damaged	Clean, Sound Rind, Slightly Damaged
Salt	Uniform	Uniform	Uniform	Uniform

RESULTS

A total of 189 wheels of Swiss were made under the observation of the laboratory representatives. Occasionally a cheese was sold before it could

be analyzed; this resulted in a final total of 176 wheels graded and analyzed. The summary of the analyses of the various samples is presented in table 2. Each sample represents the contents of a single kettle of milk, weighing approximately 2700 pounds, or its equivalent in whey, curd, or cheese.

TABLE 2

Statistical summary of analyses from twenty Swiss cheese factories in southern Wisconsin

MATERIAL	NUMBER OF SAMPLES*	AVERAGE	PLACE AND TIME OF SAMPLING
WHOLE MILK			
Per cent fat	189	3.19 ± 0.010	Sampled as the milk left the receiving vat to be clarified
Per cent casein	189	2.3 ± 0.006	
Ratio,—Casein ÷ fat	189	0.72 ± 0.002	
STANDARDIZED MILK			
Per cent fat	189	2.8 ± 0.009	Sampled in the cheese kettle just before adding rennet
Per cent casein	187	2.3 ± 0.006	
Ratio,—Casein ÷ fat	187	0.815 ± 0.003	
WHEY			
Per cent fat	188	0.90 ± 0.008	Sampled in cheese kettle at dipping
CURD			
Per cent fat	189	25.0 ± 0.07	Sampled in cheese kettle at dipping
Per cent dry matter	185	56.9 ± 0.08	
Per cent fat in dry matter	185	43.8 ± 0.10	
CHEESE			
Per cent fat	176	27.4 ± 0.06	Sampled at factory at time of grading
Per cent dry matter	177	60.4 ± 0.05	
Per cent fat in dry matter	176	45.1 ± 0.09	

* Each sample represents 2500 to 3000 pounds of mixed milk or its equivalent in whey, curd or cheese.

Composition of the whole milk delivered to the Swiss cheese factories

Fat.—Farmers in the Swiss producing area of Southern Wisconsin have never universally accepted the fat content of milk as a basis of payment. Returns are calculated commonly on the weight of milk delivered to the factory by the patrons. Breeding for production, rather than fat content, is undoubtedly responsible for the fact that the average fat content of the mixed milk delivered to many Swiss factories is actually less than 3 per cent during part of the year. Table 2 indicates an average fat content in the milk of 3.19 per cent. Figure 1 shows the distribution of the samples according to fat content.

Casein.—The average casein content of 2.3 per cent is slightly higher than might be expected with milk of this average amount of fat. The limit

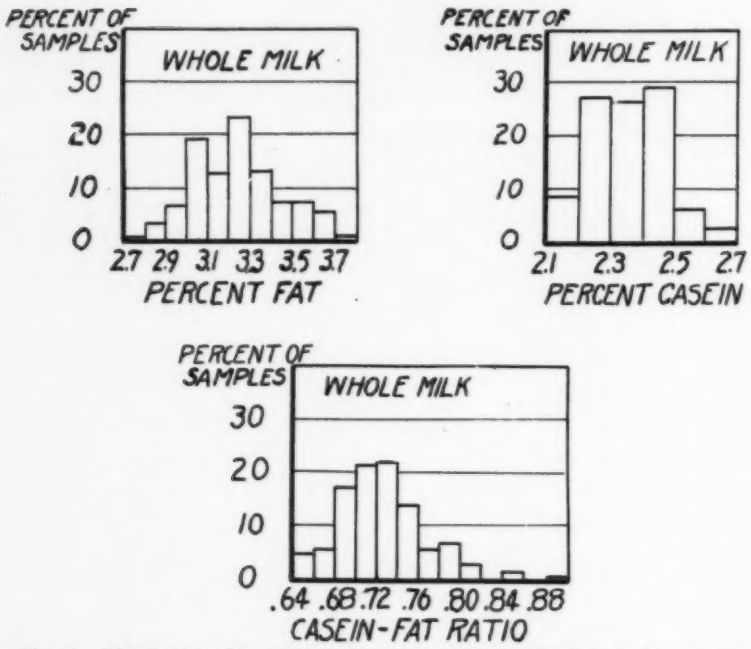


FIG. 1. Distribution of samples of whole milk classified according to the percentage of fat, percentage of casein and the ratio of casein to fat $\left(\frac{\text{casein}}{\text{fat}}\right)$.

of accuracy of the Walker test for casein, however, does not make this difference of any significance.

Ratio of casein to fat.—The ratio of casein to fat is calculated by dividing the percentage of casein by the percentage of fat in milk. Table 2 shows an average ratio of 0.72 for the milk delivered by the farmers. The highest value of this ratio observed was 0.89 per cent. It is astonishing but serves to emphasize the nature of the milk which is being used. Figure 1 illustrates the variability of the casein-fat ratio.

Composition of the standardized milk used in making Swiss cheese

It is known (5, 6) that the ratio of casein to fat in milk used for Swiss cheese has an influence on the formation of eyes. A high ratio produces better eye-forming properties in the curd. The adjustment of this ratio is called "standardization" and it is always accomplished by the removal of cream from the milk. The legal requirement of 45 per cent of fat in the dry matter of the cheese places a limit upon the extent of this adjustment.

The common factory practices for processing and standardizing the

milk in preparation for cheesemaking should be mentioned. The whole milk, as soon as it is delivered by the farmers, runs or is pumped to the separator or clarifier. Clarifiers are not used ordinarily, although one was found in a factory included in this study. The usual separator in the Swiss factory does triple duty: it clarifies the milk, removes a portion of the fat at the same time, and later in the day is used to separate the fat from the whey. The separator serves as a clarifier by simply turning both cream and skimmilk spouts into a conductor trough which carries the mixture of milk and cream to the cheese kettle. The cream is diverted from the kettle long enough to establish the desired ratio of casein to fat in the kettle milk. The regulation of this removal of cream cannot be accurate, because it is commonly based neither on accurate tests of cream or milk for fat and casein, nor upon exact knowledge of the weight of the milk in the kettle, although sometimes the depth of skimmilk in the kettle may be measured before the whole milk is allowed to run into it.

Figure 2 presents a graphical summary of the analyses of the samples of standardized milk for fat and casein, and shows the calculated casein-fat ratio.

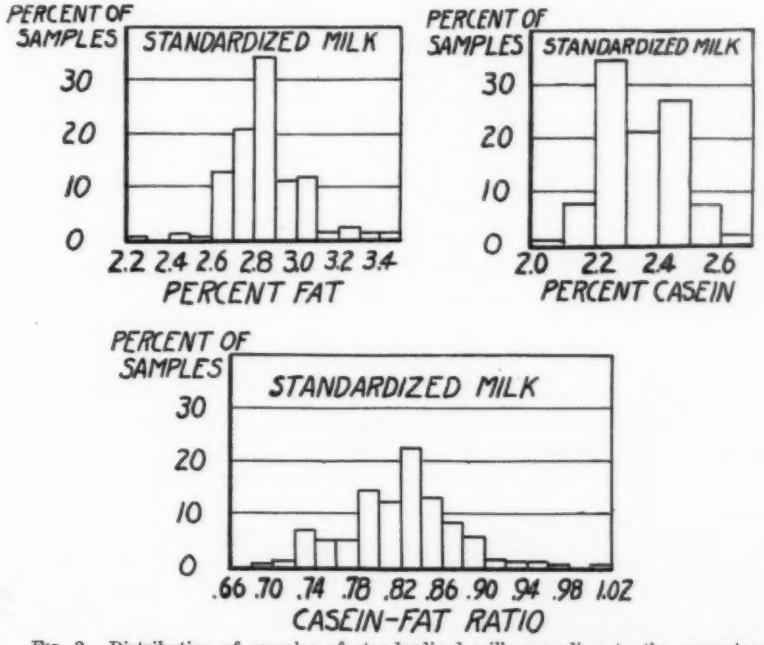


FIG. 2. Distribution of samples of standardized milk according to the percentage of fat, percentage of casein and the ratio of casein to fat $\left(\frac{\text{casein}}{\text{fat}}\right)$.

Fat.—There is a common belief among makers that a fat content of 2.8 per cent in the kettle milk makes good cheese. This may explain why the average of the samples approximates this value in table 2. Actually, however, the relative variability of the per cent fat in the standardized milk is practically the same as that of the fat in the whole milk.

Casein.—There is no measurable difference in the average casein content of the standardized milk and the whole milk, as is shown in table 2.

Ratio of casein to fat.—There is not a great deal of information available to indicate the most desirable ratio of casein to fat to establish in order to make cheese of high quality and legal composition. There are so many variable factors involved in the making process that perhaps specific recommendations are not justified. Matheson (5) recommends a ratio of casein to fat of 0.72 when the fast loss in the whey equals 0.9 to 1.0 per cent, and a ratio of 0.80 when the fat loss is 0.6 to 0.7 per cent. Peter (6, page 120) indicates that in general the cheese will contain 45 per cent of fat in the dry matter if the proportions of casein to fat are kept within the range of 0.73 to 0.75 depending upon the season of the year. He assumes that a normal loss of fat in the whey is 0.5 per cent.

In view of these recommendations it is interesting to observe in table 2 that the average ratio of casein to fat in the standardized milk is 0.815.

The data of this study indicate that the ratio of casein to fat in the standardized milk is related to the fat in the dry matter of the cheese by the correlation coefficient of $-.38 \pm .04$. Similar calculations show a correlation coefficient of $.20 \pm .05$ between the fat content of the standardized milk and the fat in the dry matter of the finished product. Evidently the ratio of casein to fat in the kettle milk is more important in determining the fat in the cheese than the actual fat content of this milk. The fat in the milk would be more definitely related to the fat in the cheese were it not for the fat losses which take place in the whey.

Loss of fat in the whey at the time of dipping

The average loss of fat in the whey at the time the curd was removed from the kettle is 0.90 per cent. Despite the wide range of the observations, indicated by the maximum and minimum values in figure 3, there are only about 1/5 of the observations which differ from the average by more than 0.2 per cent.

Although the fat losses in the whey must be a major factor in determining the fat content of the finished cheese, still, calculations indicate that the loss of fat in the whey is related to the fat content of the cheese and to the fat in the dry matter of the cheese by correlation coefficients of approximately $-.25 \pm .05$. This does not mean that excessive losses of fat may not cause the production of cheese with low fat content. As a matter of fact,

only 8 of the 22 wheels of cheese made with fat losses in the whey exceeding 1.0 per cent, contained the minimum fat required by law.

The apparently insignificant effect of the fat losses in the whey upon the composition of the cheese may be explained by the fact that the fat losses in the whey increase as the fat content of the standardized milk increases. This relationship, which is expressed by the correlation coefficient of .58 ± .03, is illustrated in figure 3. But, as the fat content of the standardized milk increases, the fat content of the finished cheese shows only a very slight tendency to increase. The correlation between these two variables is

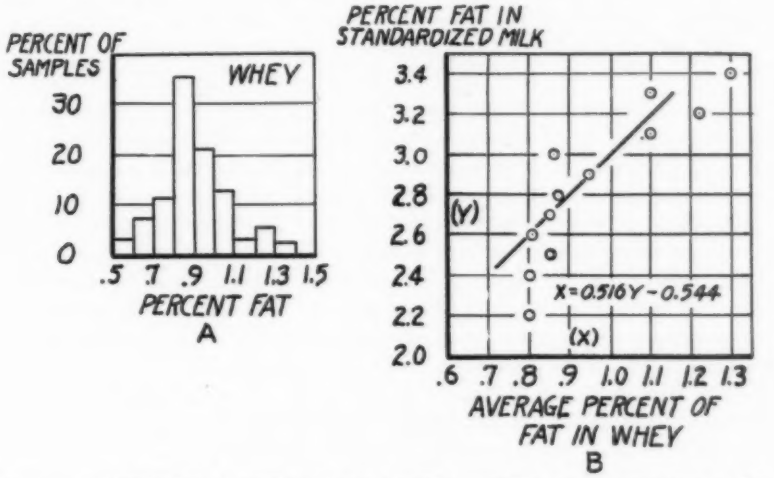


FIG. 3. A. Distribution of samples of whey classified according to the percentage of fat. B. Classification of the samples to show the average percentage of fat in the whey associated with unit changes in the fat content of the standardized milk.

only .21 ± .05. Evidently, the escape of larger and larger amounts of fat in the whey, as the fat in the kettle milk increases, reduces the amount of fat available for the cheese to such an extent that the richer standardized milk actually fails to produce any marked increase in the fat content of the cheese.

The tendency toward higher fat losses in the whey with increasing amounts of fat in the standardized milk is undoubtedly exaggerated by the so-called "clarification" of the milk with separators. The treatment is more severe than clarification in its effect on the physical condition of the fat in the milk. When milk is processed in a clarifier, the fat which gathers at the center of the bowl, is redispersed in the skimmilk as it leaves the centrifuge. Milk, which has been properly clarified, can be made into cheese

without increasing the whey fat losses that would occur if the milk had not been clarified. (7). It is commonly known, however, that cheese made from skimmilk or partly skimmed milk to which cream has been added, shows a greater whey fat loss than would occur with whole milk of the same fat content. This loss increases as the proportion of cream in the mixture increases. It is not surprising, therefore, to discover fat losses in whey from Swiss cheese approximating 0.9 per cent, under the conditions of processing which have been described. The rapidity with which the observed losses of fat in the whey increase with increasing amounts of fat in the kettle milk is undoubtedly caused by partial separation followed by inadequate mechanical redispersion of the fat in the milk.

Fat and dry matter in the curd at the time of dipping

The samples of curd were taken from the kettle and were analyzed several hours later in the central laboratory. During this interval some whey drained from the curd. Since whey contains in solution approximately 6 per cent of dry matter not fat in the form of milk sugar, milk salts, and whey-soluble proteins, the proportion of fat in the dry matter, as well as the actual percentage of fat and dry matter, were changed by this uncontrolled loss of whey from the curd. This condition, as well as those normal differences in making procedures which exist in different factories, make it impractical from our data to attempt to predict the composition of the finished cheese from the analyses of the curd at dipping.

The average values of fat, dry matter, and percentage of fat in the dry matter of the curd, are summarized in table 2 merely to complete this record.

Fat and dry matter in the cheese at the time of grading

Fat.—The fat in the cheese averages 27.4 per cent. The variations in these observations are shown in figure 4. There is a general tendency for the fat in the cheese to increase as the ratio of casein to fat in the standardized milk decreases, as indicated by the correlation coefficient of $-.35 \pm 0.04$.

Dry matter.—The dry matter in the cheese averages 60.4 per cent. Practically 85 per cent of all the samples of cheese contain within 1 per cent of this amount of dry matter, as illustrated in figure 4.

The dry matter in the cheese depends upon those factors in the making process which affect the moisture content and the retention of fat in the curd. During the curing period, the amount of salting, the humidity and temperature of the rooms, and the length of time of holding influence the dry matter in the ripened cheese. Correlations calculated between dry matter in the cheese and such factors as milk composition and loss of fat in the whey were all insignificant.

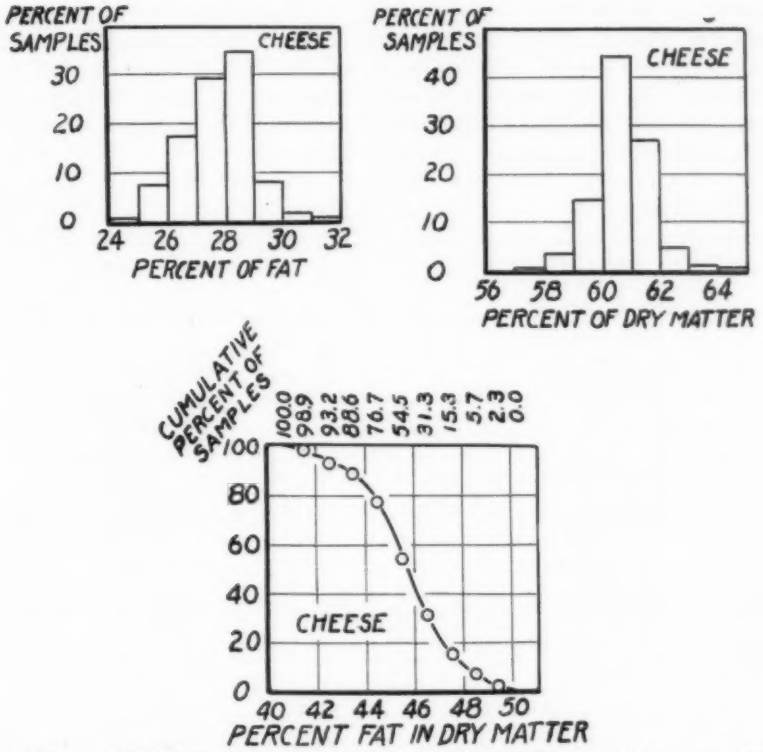


FIG. 4. Distribution of samples of cheese classified according to the per cent fat, per cent dry matter and per cent fat in the dry matter.

Fat in the dry matter of the cheese.—The percentage of fat in the dry matter of the cheese is calculated by dividing the percentage of fat by the percentage of dry matter and multiplying the result by 100. This factor is especially significant because it is used to define the legal composition of cheese. Figure 4 illustrates the variations in this calculated value for 176 wheels of cheese. This cumulative frequency distribution of the samples according to the percentage of fat in the dry matter of the cheese, indicates the proportion of the total number of samples which contain amounts of fat in the dry matter equal to or greater than any selected value shown on the horizontal axis. If, for example, 45 per cent is the chosen value, it is apparent that approximately 66 per cent of the samples contain at least this amount of fat.

The average percentage of fat in the dry matter, as shown in table 2, is 45.1. The standardization of the milk appears to be closely related to this

variable. As the ratio of casein to fat in the standardized milk decreases there is a fairly definite trend toward a higher percentage of fat in the dry matter of the cheese. This trend is expressed by the correlation coefficient $-.38 \pm .04$ and is illustrated in figure 5, in which the ratios of casein to fat in the kettle milk have been averaged for each unit change in per cent of fat in the dry matter of the cheese.

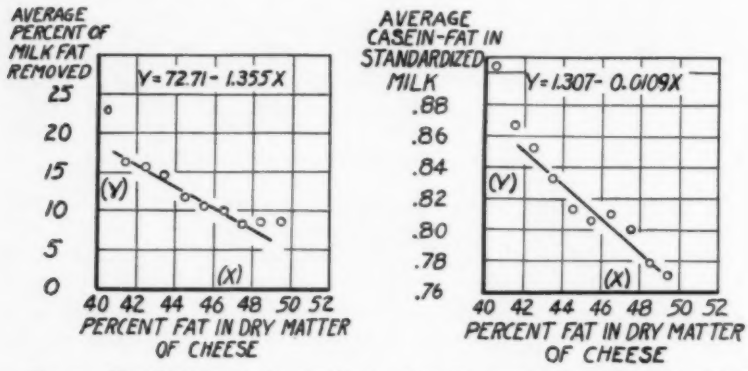


FIG. 5. Classification of the samples to show the average percentage of milk fat removed by standardizing and the average ratio of casein to fat $\left(\frac{\text{casein}}{\text{fat}}\right)$ in the standardized milk that are associated with unit changes in the percentage of fat in the dry matter of the cheese.

Another indication of the amount of fat removed by standardizing the milk has been calculated for each kettle by taking the difference between the fat tests of the whole milk and the standardized milk and dividing this difference by the per cent of fat in the whole milk. The result, when multiplied by 100, might be regarded as "The percentage of milk fat removed by standardizing." Since the exact weights of the milk in the kettle and the cream removed from the kettle were not known, this calculation is obviously inaccurate. It may serve, however, to indicate the relative amount of skimming which took place in the factories and its effect on the composition of the finished cheese. This relationship, which is described by the coefficient of correlation $-.46 \pm .04$, is shown in figure 5 where the average "percentage of milk fat removed by standardizing" has been plotted for each unit change in the per cent of fat in the dry matter of the cheese.

Figure 5 indicates the average values of the ratio of casein to fat in standardized milk and "percent of fat removed," which are associated with certain amounts of fat in the dry matter of the cheese. The application of these values to determine adjustments of milk composition at the beginning of the making process must be attempted with caution. These values are

averages for many plants and the observations upon which they are based were made over several months. They only indicate the trends of the effects and dangerous limits of adjustments of milk composition. It seems reasonable to believe that the variability of the data from all the plants is greater than that which should obtain for similar measurement in any single plant where manufacturing conditions are more uniform from day to day. Careful measurements repeated each day in any given factory, therefore, should make it possible to establish more definite relationships than those indicated in figure 5.

The quality of the cheese

The average of the grades of the lots of cheese produced during the study is 2.16.

Coefficients of correlation calculated between the grade and such variables as: fat in the milk delivered by the farmers, casein-fat ratio in standardized milk, and fat loss in the whey, any of which might be expected to influence the grade of the cheese, were all reasonable in trend but insignificant in value. As each factor tended to produce or to indicate a decrease in the percentage of fat in the dry matter of the cheese there was a slight tendency toward improvement in the quality of the cheese. The fat in the dry matter of the cheese is the only observed variable which seems to influence the grade. This relation is summarized in table 3 in which the average percentage of fat in the dry matter has been calculated for the cheese falling in each grade. As the grade moves from I to IV there is

TABLE 3

Relation between grade and percentage of fat in the dry matter of cheese

GRADE OF CHEESE	NUMBER OF CHEESE	AVERAGE PER CENT FAT IN DRY MATTER OF CHEESE
I	68	44.5
II	49	44.8
III	23	45.1
IV	36	46.7
Total	176	

a gradual increase in the average fat content of the cheese. This relationship is described by the coefficient of correlation $.42 \pm .04$.

DISCUSSION

The graders of Swiss cheese act essentially as the interpreters of trade demands because they are employed by those who market the cheese. The premium paid for the highest quality of Swiss cheese is attractive enough to determine the trends of factory practices. Since the best grades seem to be associated with less fat in the dry matter of the cheese it is natural to expect the makers to reduce the fat in the finished product to the minimum.

Although trade demands are rightfully interpreted by those who merchandise Swiss cheese, it is reasonable to question the influence on the dealers' opinions of the demands of the large buyers who desire cheese with good slicing properties and large eye formation. A survey of the industry and public opinion would be necessary to determine whether these characteristics are more important to the ultimate consumer than the mellowness of body and superior flavor which can be best attained by retaining a larger proportion of the milk fat in the cheese.

There are other factors, outside the scope of this investigation, that influence, directly or indirectly, the composition and quality of the cheese, such as: milk quality, the use of milk from diseased udders, and the culture methods of controlling fermentations in the cheese. It is not known how much improvement in market grades is possible by regulating these factors and maintaining at the same time a mechanical control of cheese composition which would incorporate maximum amounts of fat. Information obtained by the cooperative efforts of the United States Bureau of Dairy Industry and the University of Wisconsin during the past several years indicate that milk of better quality and application of improved methods of using the available cultures definitely raise the market quality of the cheese.

It is evident, therefore, that any program of composition regulation, especially when such regulation must raise the fat content of the finished cheese, should also be accompanied by increased efforts to control the quality of the milk and the types of organisms in the cheese.

CONCLUSIONS

Mechanical control of the fat content of Swiss cheese depends upon accurate standardization. Simultaneous clarification and separation of milk, excessive losses of fat in the whey, and milk with abnormally low fat content makes such standardization a difficult procedure in some plants.

The removal on the average of more than about 10 per cent of the total fat from the lots of milk observed in this study or the establishment of casein to fat ratios greater than approximately 0.81, tend to produce cheese with less than the legal amount of fat. Since unusual biological conditions in the milk or special methods of curdmaking may cause variations in cheese composition, it is essential that every plant operator analyze the milk, whey, and cheese from every kettle before accepting these average values as actual standards.

REFERENCES

(1) SHERMAN, JAMES M. The cause of eyes and characteristic flavor in Emmental or Swiss cheese. Jr. Bacteriology 6: page 379. 1921.

(2) SHERMAN, J. M. The use of bacterial cultures for controlling the fermentation in Emmental cheese. Proceedings of World's Dairy Congress 1: page 287, 1923.

(3) WATSON, PAUL D. The relation of the hydrogen-ion concentration to the texture of Emmental or Swiss cheese. JOURNAL DAIRY SCIENCE 12: page 289. 1929.

(4) FRAZIER, W. C., BURKEY, L. A., MATHESON, K. J., AND WATSON, P. D. Thermophilic streptococci as starters for Swiss cheese. JOURNAL DAIRY SCIENCE 16: page 387. 1933.

(5) MATHESON, K. J. New developments in the manufacture of Swiss cheese. Proceedings of the World's Dairy Congress 1: pages 290–296. 1923.

(6) PETER, A. Praktische Anleitung zur Fabrikation und Behandlung des Emmentalerkäses. K. J. Wyss Erben, Bern, Switzerland. 1930.

(7) FISK, WALTER W., AND PRICE, WALTER V. The clarification of milk for cheese making. Cornell University Agricultural Experiment Station, Ithaca, N. Y. Bulletin 418. 1923.

RUMINANT DIGESTION WITHOUT ROUGHAGE

S. W. MEAD AND HAROLD GOSS

Division of Animal Husbandry, University of California, Davis, California

In a study of the value of roughage in the dairy ration, several heifers have been maintained at this station on a roughage-free diet from birth to over 18 months of age. A similar group was reared on the same mixture of concentrates plus approximately 14 per cent of sulphite wood paper pulp. During this work, digestion trials were conducted with heifers from each group. The results are here reported.

PROCEDURE

Three digestion trials were conducted, four heifers being used in each. The animals received the same feed mixtures that they had been receiving since two months of age except for the mixture used in trial 3. During this trial and for ten days before the collection period, they received mixture 3, which differed from mixture 1 only in being more finely ground. This trial was intended to determine the effect of fine grinding on digestibility: mixture 2 was more finely ground than mixture 1, because of the method of preparation necessary to incorporate the paper pulp. The heifers received daily a medicinal cod liver oil, the total amount of which is shown in table 2. The compositions of the feed mixtures are shown in table 1.

TABLE 1

Percentage composition of feed mixtures

FEED MIXTURE NO.	INGREDIENTS						
	Ground barley	Wheat bran	Soybean meal	Sodium chloride	Calcium carbonate	Calcium citrate	Paper pulp
1	61.5	26.3	8.8	1.9	1.5	.....	.....
2	52.0	22.2	7.4	1.6	.....	3.0	13.8
*3	61.5	26.3	8.8	1.9	1.5	.....	.....

* Identical in composition with feed mixture 1 but more finely ground.

The animals used were grade Holstein heifers, 18 months of age, which had received since birth a ration devoid of roughage. They were normal in size for the breed and showed no abnormalities other than frequent bloating and lack of regular ruminations, both of which have been found to be common with ruminants receiving this type of ration. According to analysis, the gas causing bloat consisted largely of carbon dioxide with no methane.

During the digestion trials the daily amounts of feed for each animal

Received for publication October 10, 1934.

were reduced, since animals usually consume less when standing in stanchions than when exercising in the corral.

The heifers were placed in the digestion stalls 6 days before the collection of feces. The collection period lasted for 10 days. Heavy gymnasium mats replaced the common forms of bedding. The animals were fed and watered thrice daily. Each day, the total feces collected during the previous 24 hours were spread out in drying trays and placed in a drying oven maintained at a temperature of 90° to 95° C.[1] A period of 48 hours was required to dry each day's collection. When dry, the feces from each animal were placed in separate tightly covered cans. At the end of the collection period the dried feces were weighed, and a sample was taken for moisture. The remainder was ground in a hammer mill, thoroughly mixed, and sampled for chemical analyses.

PRESENTATION OF DATA

The amounts of feed consumed and feces collected are shown in table 2. Chemical analyses of feed mixtures and feces are presented in tables 3 and 4; coefficients obtained, in table 5.

TABLE 2
Total food consumed and feces collected (grams)

ANIMAL NO.	TRIAL 1			TRIAL 2			TRIAL 3		
	Mixture 1	Cod liver oil	Feces (dry weight)	Mixture 2	Cod liver oil	Feces (dry weight)	Mixture 3	Cod liver oil	Feces (dry weight)
34	40,800	558	8,220	.....	.....	.....	36,300	558	7,180
35	58,860	1116	10,870	.....	.....	.....	43,800	1116	9,560
37	45,400	558	8,740	.....	.....	.....	40,800	558	9,010
39	54,400	558	11,050	.....	.....	.....	45,400	558	10,710
41	.....	.....	.....	56,410	630	15,200	.....	.....	.....
43	.....	.....	.....	40,800	558	8,160	.....	.....	.....
46	.....	.....	.....	36,300	558	10,410	.....	.....	.....
47	.....	.....	.....	36,300	558	7,580	.....	.....	.....

During the three digestion trials all but two of the animals cleaned up their feed well. Number 35 left 137 grams of the last feeding of trial 1; and during trial 3, when receiving the finely ground feed, she had an irregular appetite and refused a total of 1,600 grams. Number 41 left 2,590 grams of feed during trial 2. The daily ration of mixture 3 (table 2) was less than had been allowed the same animals in trial 1, in anticipation of a decreased consumption. While receiving this finely ground feed before the

[1] While this paper was in preparation, D. P. Cuthbertson and A. K. Turnbull, (Biochem. J. 28: 837, 1934) found a loss of 4 to 5 per cent of nitrogen in drying human feces on the steam bath. In our laboratory, R. W. Caldwell has confirmed these findings: The nitrogen loss was 3 to 4½ per cent in drying freshly voided bovine feces at 100° C., for 48 hours in an electric oven.

TABLE 3
Chemical analyses of feed mixtures, per cent

	TRIAL 1		TRIAL 2*				TRIAL 3	
ANIMAL NO.	34–35	37–39	41	43	46	47	34–35	37–39
Moisture	10.3	10.7	9.0	9.0	9.5	8.4	11.8	11.1
Protein	14.7	14.0	12.0	12.2	12.3	12.2	13.9	13.9
Nitrogen-free extract	60.0	61.0	54.9	56.1	55.4	56.1	60.3	60.4
Ether extract	1.3	1.4	0.7	0.7	0.8	0.8	1.3	1.3
Crude fiber	6.9	6.5	17.6	16.3	16.1	16.7	6.5	7.2
Ash	6.8	6.4	5.8	5.7	5.9	5.8	6.2	6.1

* Because of the physical nature of mixture 2, separate samples of ths feed were analyzed for each animal.

TABLE 4
Chemical analyses of feces collected, per cent dry basis

	TRIAL 1				TRIAL 2				TRIAL 3			
ANIMAL NO.	34	35	37	39	41	43	46	47	34	35	37	39
Protein	13.2	12.7	14.8	12.4	10.2	13.3	11.5	11.2	12.7	11.7	12.4	12.3
Nitrogen-free extract	47.0	45.2	45.9	46.4	38.5	44.1	42.9	44.0	47.8	48.5	48.6	47.4
Ether extract	2.5	3.3	2.7	2.6	2.4	2.3	3.0	2.5	3.0	2.2	2.9	2.6
Crude fiber	20.6	20.8	21.0	22.4	38.9	27.9	31.4	29.8	22.0	23.5	23.0	23.3
Ash	16.7	18.0	15.6	16.2	10.0	12.4	11.2	12.5	14.5	14.1	13.1	14.4

TABLE 5
Coefficients of digestibility

	DRY MATTER			PROTEIN			NITROGEN-FREE EXTRACT			ETHER EXTRACT			CRUDE FIBER		
TRIAL NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Animal No.															
34	78	.....	78	82	.....	82	84	.....	84	81	.....	79	40	.....	33
35	80	.....	76	84	.....	82	86	.....	82	81	.....	88	44	.....	21
37	79	.....	76	80	.....	80	86	.....	82	80	.....	76	38	.....	30
39	78	.....	74	82	.....	79	85	.....	82	78	.....	76	30	.....	24
41	.....	71	.....	.....	77	.....	.....	81	.....	.....	64	.....	.....	40	.....
43	.....	78	.....	.....	78	.....	.....	84	.....	.....	78	.....	.....	66	.....
46	.....	69	.....	.....	73	.....	.....	78	.....	.....	63	.....	.....	44	.....
47	.....	78	.....	.....	81	.....	.....	84	.....	.....	78	.....	.....	63	.....
Average	79	74	76	82	77	81	85	82	83	80	71	80	38	53	27
Calculated	*82	.....	.....	86	.....	.....	88	.....	.....	81	.....	.....	56	.....	.....

* See text page 6.

digestion trial, the heifers apparently found it less palatable than the same mixture coarsely ground. Their feces were drier than with the coarsely ground mixture 1. A dose of one-half pound of Epsom salts had to be administered to heifer 35 on the fourth day of the collection period; thereafter her appetite improved somewhat, so that she cleaned her feed up fairly well at each feeding.

During trial 1 all the animals ruminated occasionally. In trial 2, No. 41 and No. 43 ruminated, but not regularly. No. 46 and No. 47, receiving less feed, were not observed to ruminate at any time. During trial 3, with the finely ground feed, no rumination was noticed.

The paper pulp was originally added to the basal concentrate mixture to serve as an artificial roughage, somewhat less complex than natural roughage. Rumination occurred only occasionally, however, no more in one group than another, even though concentrate mixture 1 contained 6.8 per cent crude fiber, whereas the same mixture plus paper pulp, mixture 2, contained 16.7 per cent.

TABLE 6

Water consumption and number of defecations and urinations during each ten-day collection period

ANIMAL NO.	DEFECATIONS	URINATIONS	WATER CONSUMED (POUNDS)
		Digestion Trial 1	
34	63	104	350
35	44	58	483
37	68	73	330
39	63	70	332
Average	60	76	374
		Digestion Trial 2	
41	76	36	350
43	76	55	251
46	49	27	201
47	50	46	208
Average	63	41	253
		Digestion Trial 3	
34	59	92	330
35	49	61	200
37	66	65	351
39	78	69	308
Average	63	72	297

The amounts of water consumed, with the number of defecations and urinations, are reported in table 6. During a ten-day metabolism trial in connection with another experiment, nine cows receiving a normal ration of concentrates and hay averaged 11 defecations and 5 urinations daily. In

these cases there was a variation of 9 to 15 defecations and 3 to 7 urinations. The number of defecations was almost double that recorded for the heifers used in the digestion trials here reported; the number of urinations, about the same.

DISCUSSION

As roughage has been considered essential in the diet of ruminants, the accepted procedure for determining the coefficient of digestibility for a given concentrate has been first to determine the digestibility of a roughage, which is later fed in combination with the concentrate, second to determine the digestibility of the combination ration, and third to calculate the difference between the two. Whether or not the results so obtained would be similar to those for the concentrate if fed alone has been considerably debated, though the usefulness of the information is not questioned.

The literature describes several investigations that have increased our knowledge regarding the effect of one feed or nutrient upon another. Since Titus (1), in discussing "the mutual influence of the proportion of the several nutrients, in feeds, on their digestibility," has reviewed this literature very completely, only investigations particularly pertinent to this discussion will be mentioned here.

We have failed to find any data concerning the digestibility of concentrates when fed without roughage for an extended period. In the investigations conducted by others, the concentrates were fed alone only for a relatively short time, doubtless because roughage has been considered essential. As experiments at this station (2) (3) have shown, however, dairy animals can be reared to four years of age on a concentrate diet provided it is supplemented with calcium carbonate and vitamin A.

According to certain evidence, the digestibility of a diet may vary with the length of time it is fed. This might be especially true should the diet be unusual—for example, composed entirely of concentrates. In this connection the work of Lyman (4) is interesting. He has shown that the continued use of white flour, in the diet of rats, caused a slight decrease in protein utilization. The continued use of whole wheat meal in a similar food combination, however, decidedly improved the utilization of this nutrient.

The coefficients of digestibility determined for mixture 1 (concentrates only) have been compared in table 5 with calculated values for this mixture when fed with roughage, arrived at by using the coefficients of each ingredient in the mixture as given by Henry and Morrison (5). The coefficients of digestibility for the dry matter and the ether extract of cod liver oil were both assumed to be 90 per cent, that of the dry matter of soybean-oil meal 92 per cent, and that of salt 100 per cent. The digestibility of calcium carbonate was disregarded. Except in the case of crude fiber,

which was 32 per cent lower, the coefficients actually determined were not significantly below those calculated.

Admittedly, the coefficients of digestibility used in calculating values are based upon a relatively few trials; and nutrients existing in comparatively small amounts, such as the crude fiber in our mixture 1, may give erroneous results. The heifers used in trial 1, however, did not ruminate regularly, a fact that may explain the apparent low digestibility of the crude fiber. As Ewing and Wright (6) have shown "More than half of the comminution that takes place in average rations containing coarse feeds takes place as a result of mastication." In an earlier publication, Ewing, Wells, and Smith (7) showed that a steer would macerate his food more completely when fed silage alone than when fed silage and cottonseed meal, the extent of maceration decreasing as the proportion of meal increases; and that the loss in digestibility of the crude fiber, which resulted from a combination of silage and cottonseed meal, was caused by a less complete maceration.

Mixtures 1 and 3 were identical except that the latter was more finely ground. The digestibility of mixture 3 was determined in order that the results obtained for mixtures 1 and 2 would be on a more comparable basis, since mixture 2 was necessarily more finely ground than mixture 1. In this trial (table 5), mixtures 1 and 3 showed no significant difference in digestibility except in crude fiber. Not only was the average coefficient of digestibility for this ingredient in mixture 3 lower, but none of the animals showed so high a value for this nutrient in trial 3 as in trial 1. The results, therefore, appear significant. This decrease might be explained by the work of Ewing and Smith, (8) who, having studied the rate of passage of food residues through the steer, concluded that finer-ground feeds pass through the animal more rapidly and that the digestibility of crude fiber seems to decrease with a more rapid passage of feed residues.

According to trial 3, any differences in digestibility between mixtures 1 and 2, except for crude fiber, cannot be explained on the basis of fine grinding.

When the coefficients of digestibility for mixture 1, consisting entirely of concentrates, are compared with mixture 2, containing 14 per cent paper pulp (table 5), the digestibility of all nutrients except fiber is seen to decrease slightly. These decreases, however, are probably not significant.

The digestibility of the crude fiber in mixture 2 was considerably higher than that in mixture 1 and approximately the same as the calculated value for mixture 1 when fed with roughage, doubtless because paper pulp itself has a high coefficient of digestibility. In two trials with oxen, Kellner (9) found the digestibility of crude fiber in paper pulp obtained by chemical treatment of rye straw to be 94.7 and 97.0 per cent. To explain his results he calls attention first to the fineness of the fibers in the paper pulp and

second to the fact that the chemical treatment had removed the incrusting substances which normally interfere with its digestion.

The digestibility of the crude fiber in the sulphite pulp used in our trials, calculated indirectly from our data, was lower than Kellner reported, but higher than the digestibility of the crude fiber in hays.

As the paper pulp in mixture 2 represented two-thirds of the fiber content of this mixture, the increased digestibility of the fiber in mixture 2 can be explained by the high digestibility of the paper pulp fiber

SUMMARY

Digestion trials were conducted with Holstein heifers that had been fed for 18 months on a ration devoid of roughage, and with a second group receiving the same diet plus paper pulp. The effect of fine grinding on digestibility was also investigated.

Except for crude fiber, which was 32 per cent lower, the digestibility of a ration consisting entirely of concentrates was not significantly below the calculated value for the same mixture fed with roughage.

The addition of paper pulp to the concentrate diet, so that the fiber content of the ration was equivalent to that of one containing equal parts of concentrates and alfalfa hay, increased the apparent digestibility of the crude fiber. The digestibility of the other nutrients did not appear to be significantly altered.

It is not to be concluded that the addition of paper pulp enhanced the digestibility of the fiber contained in the concentrates. The higher value found for fiber in the paper-pulp ration may be explained by the fact that the fiber of paper pulp, as shown by other investigators, is more highly digestible than that of either roughage or concentrates.

Fine grinding appeared only to lower significantly the digestibility of the crude fiber of the concentration ration.

REFERENCES

(1) Titus, Harry W. The mutual influence of the proportion of the several nutrients, in feeds, on their digestibility. I. Crude fiber—The digestibility of rations containing varying amounts of alfalfa and paper pulp. New Mexico College of Agriculture and Mechanic Arts. Tech. Bul. 153. 1926.

(2) Mead, S. W., and Regan, W. M. Deficiencies in rations devoid of roughage for calves. I. The effect of the addition of cod liver oil and alfalfa ash. Journal of Dairy Science 14: 283–293, 1931.

(3) Mead, S. W., and Goss, Harold. Unpublished data, Division of Animal Husbandry, University of California, Davis, California.

(4) Lyman, J. F. Cumulative effects on protein utilization of highly milled and whole wheat flours. Soc. Expt. Biol. and Med. Proc. 29: 156–158. 1931.

(5) Henry, W. A., and Morrison, F. B. Feeds and Feeding, tables I and II, Appendix, Eighteenth Edition. The Henry-Morrison, Company, Madison, Wisconsin, 1923.

(6) EWING, P. V., AND WRIGHT, L. H. A study of the physical changes in feed residues which take place in cattle during digestion. Jour. Agr. Res. **13**: 639–646. 1918

(7) EWING, P. V., WELLS, C. A., AND SMITH, F. H. The associative digestibility of corn silage and cottonseed meal in steer rations. Part II. The influences exerted by corn silage and cottonseed meal on the digestion co-efficients of each other when fed in combination, as determined by means of a fractional separation of the feces. Georgia Exp. Station Bul. **125**. 1917.

(8) EWING, P. V., AND SMITH, F. H. A study of the rate of passage of food residues through the steer and its influence on digestion coefficients. Jour. Agr. Res. **10**: 55–63. 1917.

(9) KELLNER, O. Reihe IV. Versuche mit Wiesenheu, Weizenstroh, Stärkemehl, extrahiertem Roggenstroh und Melasse. Landw. Vers. Sta. **53**: 278. 1900.

THE USE OF SODIUM THIOSULPHATE DILUTION BLANKS IN DETERMINING THE GERMICIDAL EFFICIENCY OF CHLORINE STERILIZERS*

C. C. PROUTY AND ROY T. OLSON

Division of Dairy Husbandry and Department of Bacteriology, State College of Washington, Pullman, Washington

Although chlorine compounds are widely used as sterilizing agents for dairy and milk plant equipment, their use is not sanctioned by all regulatory agencies nor are all investigators in agreement as to their effectiveness in destroying microorganisms.

Mudge and Smith (2) present a critical discussion of the effectiveness of chlorine compounds as dairy sterilizers and raise the question as to whether bacteria are killed or merely inhibited in growth by the action of these compounds. Furthermore, they question the value of plate counts as made by the usual laboratory methods for determining the germicidal efficiency of chlorine sterilizers. They point out that the results obtained by plate counts are a measure of the inhibiting action rather than a measure of the lethal effect of the chlorine compound. They express the opinion that, when a solution containing a chlorine compound is added to the peptone agar, the chlorine remains as a potent organic chloramine capable of inhibiting subsequent bacterial multiplication. To prevent the bacteriostatic action of residual chlorine they suggest the neutralization of this chlorine by the addition of sodium thiosulphate to the plate at the time of plating. They obtained, in general, a greater colony count with plates to which sodium thiosulphate had been added then with plates not receiving this treatment.

In addition to demonstrating the neutralizing effect of sodium thiosulphate, Mudge and Smith present evidence to show the effect of dilution of the residual chlorine carried over into the plate on the reduction of the bacteriostatic action. They report a colony count many times higher in the 1–10 than in the 1–1 dilution. They found this phenomenon to be the rule whether they used hypochlorite or monochloramine in high or low concentrations and with or without additions of sodium thiosulphate.

Devereux and Mallman (1) present data to show the value of sodium thiosulphate in determining the effectiveness of chlorine sterilizers against organisms in dairy equipment. Additions of sodium thiosulphate at the time of sampling neutralizes the chlorine thus making it possible to ascer-

Received for publication October 23, 1934.

* Published as Scientific Paper No. 248, College of Agriculture and Experimental Station, State College of Washington.

tain the true bacteriological status of the solution at the time of sampling even though immediate plating is not possible.

In studying the germicidal efficiency of a disinfectant it is necessary that the period of exposure of the disinfectant to the test organism be under definite control. Methods that do not permit of such a control because of the bacteriostatic action of the residual disinfectant are likely to give misleading results. In making plate counts of chlorine solutions the most desirable place to neutralize the residual chlorine is in the dilution blank. Accordingly the present investigation was planned to determine to what extent the presence of sodium thiosulphate in the dilution blank is of value in preventing the bacteriostatic action of the chlorine as shown by the subsequent colony count. Studies also were made of the effect of dilution in reducing the bacteriostatic action of the residual chlorine as shown by the numbers of colonies developing in plate cultures made from inoculated chlorine solutions.

METHODS

Dilution blanks of convenient volumes containing sodium thiosulphate were prepared as follows: To 50 cc. of M/5 Na_2HPO_4 was added 9.0 cc. of N HCl and the solution made up to 200 cc. with distilled water. To this buffered solution was added a 1.0 per cent solution of sodium thiosulphate at the rate of 10.0 cc. per 90.0 cc. after which the dilution blanks were prepared and sterilized. Preliminary experiments had shown this concentration of sodium thiosulphate in a 9.0 cc. dilution blank to be more than enough to neutralize the chlorine carried in a 1.0 cc. charge of the most concentrated chlorine solution used in this study and yet not interfere with subsequent colony growth. The resulting dilution blanks had a pH value of approximately 6.4. At this pH the reaction of the dilution blank is not deleterious to the test organisms and is favorable to the rapid reactivity of sodium thiosulphate and chlorine.

Plate cultures using plain agar were made immediately, 2.5, 5.0, 7.5, 10 and 15 minutes after inoculating the chlorine solution with the test organism. These cultures were plated simultaneously by two operators when dilution blanks with and without sodium thiosulphate were being compared. Plate cultures were incubated at 37° C. usually for a period of forty-eight hours.

Test organisms consisted of twenty-four hour old cultures of *Escherichia coli, Aerobacter aerogenes, Staphylococcus albus, Staphylococcus aureus* and *Streptococcus lactis*. Milk in which fermentation had taken place also was used.

The chlorine sterilizer used was a commercial preparation in which the active ingredient was calcium hypochlorite.

TABLE 1

Comparative plate counts of inoculated chlorine solutions made with and without sodium thiosulphate dilution blanks

P.P.M. Cl.	TEST ORGANISM	BACTERIA PER CUBIC CENTIMETER AFTER											
		Immediately		2.5 Minutes		5 Minutes		7.5 Minutes		10 Minutes		15 Minutes	
		With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
21	*Esch. coli*	43,000	47,000	40,500	10,600	7,250	550	550	35	0	0	0	0
51	*Esch. coli*	101,000	70,000	12,600	10,400	700	140	20	0	0	0	0	0
98	*Esch. coli*	10,300	12,150	95	10	0	0	0	0	0	0	0	0
38	*Staph. aureus*	4,700	2,650	115	35	10	0	0	0	0	0	0	0
59	*Staph. aureus*	40,000	45,000	5,000	5,000	1,200	1,050	120	90	0	0	0	0
33	*Staph. albus*	3,500	2,050	750	950	450	125	30	20	25	15	15	30
31	*Staph. albus*	7,750	7,700	5,250	5,850	4,300	4,700	2,800	3,300	2,350	2,350	480	50
42	*S. lactis*	1,450	2,200	1,550	600	450	350	0	0	0	0	0	0
18	*S. lactis*	5,100	6,050	500	150	300	50	250	10	0	0	0	0
63	*Aer. aerogenes*	32,000	38,000	24,000	18,800	4,200	2,400	580	24	40	0	0	0
84	*Aer. aerogenes*	21,000	24,000	4,000	2,900	80	0	0	0	0	0	0	0
22	Milk	5,450	4,400	500	160	585	40	220	0	0	0	0	0
75	Milk	72,000	68,000	21,000	7,000	12,800	420	830	0	0	0	0	0
100	Milk	44,000	38,000	11,800	4,200	800	90	80	20	40	20	10	10
40	Milk	52,000	34,000	38,000	24,000	9,500	1,800	1,100	320	60	40	20	20
60	Milk	7,200	7,200	3,900	2,900	1,200	450	0	0	0	0	0	0

PRESENTATION OF DATA

In table 1 are shown the results of sixteen comparative plate counts of inoculated chlorine solutions in which simultaneous platings were made using dilution blanks with and without sodium thiosulphate. Colony development, after brief periods of exposure of the organisms to the action of chlorine, up to 7.5 minutes and depending on the chlorine concentration, showed in most cases an increased count with the use of sodium thiosulphate in the dilution blank. However, when the organisms were exposed to the action of chlorine for periods of ten minutes or more, sodium thiosulphate had little or no effect on the subsequent colony development. The differences in numbers after brief periods of exposure to chlorine, while not great, show that bacterial cells which otherwise would be destroyed or inhibited in growth by the residual chlorine are able to continue normal development when freed of this chlorine. This is a factor that should be considered in evaluating the true germicidal efficiency of a chlorine sterilizer.

In the course of this study many plate counts were made in an effort to determine the influence of dilution on the bacteriostatic action of residual chlorine as shown by the numbers of colonies developing on plates of different dilutions. In no case was it found that more colonies developed on the plates of the higher dilutions than on plates of the lower dilutions. In this respect these results are not in agreement with those of Mudge and Smith (2).

SUMMARY AND CONCLUSIONS

Simultaneous platings of inoculated chlorine solutions immediately after inoculating and after 2.5, 5.0, 7.5, 10 and 15 minutes, using ordinary dilution blanks and dilution blanks containing sodium thiosulphate were made and compared. In most cases an increased colony count resulted when sodium thiosulphate dilution blanks were used. Colony development following brief periods of exposure of the organisms to the action of chlorine showed, in most cases, an increased count when sodium thiosulphate dilution blanks were used. After longer periods of exposure, ten or more minutes, the presence of sodium thiosulphate had little or no effect on subsequent colony development.

The results of these experiments show that plate counts, in which ordinary dilution blanks have been used are likely to be misleading in evaluating the germicidal efficiency of chlorine sterilizers.

In an effort to study the effect of dilution in the bacteriostatic effect of the residual chlorine, plate counts of inoculated chlorine solutions, using ordinary dilution blanks, were made and the numbers of colonies developing on the plates of the 1–1, 1–10 and 1–100 dilutions compared. In no instance were more colonies found to develop on the plates of the higher dilutions than on plates of the lower dilutions.

REFERENCES

(1) DEVEREUX, E. D., AND MALLMAN, W. L. Studies of the technique to evaluate the efficiency of several chlorine sterilizers for dairy use. JOURNAL OF DAIRY SCIENCE, 17: 351–360. 1934.

(2) MUDGE, C. S., AND SMITH, F. R. On the germicidal action of chlorine. Proceedings, Nineteenth Annual Meeting Western Division, American Dairy Science Association, October, 1933.

A METHOD FOR THE DETERMINATION OF THE RELATIVE STIFFNESS OF CREAM DURING THE WHIPPING PROCESS*

W. S. MUELLER

Department of Dairy Industry, Massachusetts State College, Amherst

The purpose of this paper is to describe a simple method for measuring the stiffness of cream during the whipping process and to show the importance of such measurements.

Since most consumers judge the quality of whipped cream by its consistency or stiffness, this property should be measured in studies of cream whipping. In the majority of such studies reported, manually operated whippers have been used and no provision has been made for measuring the consistency of the cream during whipping nor of the finished product. Babcock[1] describes a method for determining the relative stiffness of whipped cream. His method is not, however, applicable to uninterrupted measurements throughout the whipping process. So far as the writer knows, Templeton and Sommer[2] were first to report studies in which a mechanical whipper of constant speed was used and in which some provision was made for determining the stiffness of the cream during the whipping process. These investigators measured the consistency of the whipped cream by observing the torque (load) on the drive shaft of the turbine whipper.

DESCRIPTION OF APPARATUS

The apparatus described herein consists of the mechanical whipper (restaurant size) and sensitive wattmeter, shown in figure 1. With this apparatus it is possible to obtain a continuous record of the stiffness of the cream throughout the whipping process by simply recording the input of the motor in watts at intervals of ten seconds, or less if desired. This method for measuring the stiffness of the cream in watts cannot, however, be used with all mechanical whippers, as the writer found in preliminary experiments with several small household whippers. The writer learned also that another investigator had also attempted in vain to use the wattmeter for measuring the relative stiffness of the cream as it was being whipped. These failures, no doubt, were due to certain characteristics of the mechanical whippers used. Furthermore, in order that small changes in load will be reflected in large changes in input, a motor should be used the maximum output of which is not much greater than the maximum

Received for publication November 27, 1934.

* Contribution No. 208 of the Massachusetts Agricultural Experiment Station.

[1] Babcock, C. J. 1922. U. S. Dept. of Agric., Bul. 1075.

[2] Templeton, H. L., and Sommer, H. H. 1933. Jour. of Dairy Sci., xvi, no. 4, 330.

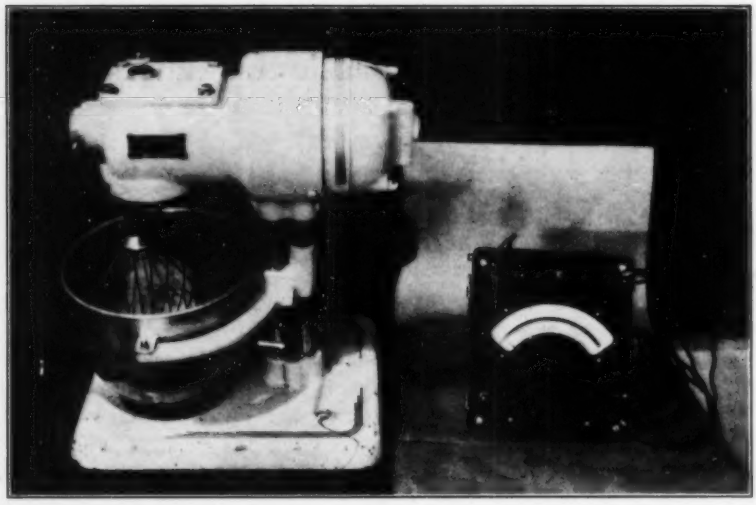


FIG. 1. Mechanical cream whipper and wattmeter. (Gem kitchen mechanic whipper manufactured by Gem Appliances, Inc., 280 Madison Ave., N. Y., and a Westinghouse type PY5 portable single phase wattmeter).

required to whip the cream. If the normal output of the motor is relatively large, and the changes in load very small, these small changes in load will be insufficiently indicated by the variations in input as measured by the ordinary wattmeter.

The whipper-motor (figure 1), of which the input is charted, is a split-phase motor rated $\frac{1}{8}$ HP and 1725 RPM. This motor produces in the whipper proper two distinct kinds of rotation. First, it spins the whipper on its vertical axis counter clockwise at a speed of 570 RPM. Second, it moves the spinning whipper (and its axis) clockwise about the inner periphery of the bowl so that the whipper completes 78 circuits about the bowl each minute. The speed of the whipper was checked under all loads which are normally encountered when whipping cream up to 40 per cent butterfat, and was found to be practically constant. Although the whipping bowl has a 5-quart capacity (liquid measure), only one quart of cream is usually placed in the bowl for whipping. The smallest scale division on the wattmeter is 5 watts, but 2.5 watts can be readily estimated.

That this wattmeter method is satisfactory to determine the relative stiffness of whipping cream at various times during whipping is proved in figure 2, in which appear typical curves obtained by plotting input against time when whipping cream of varying percentages of butterfat.

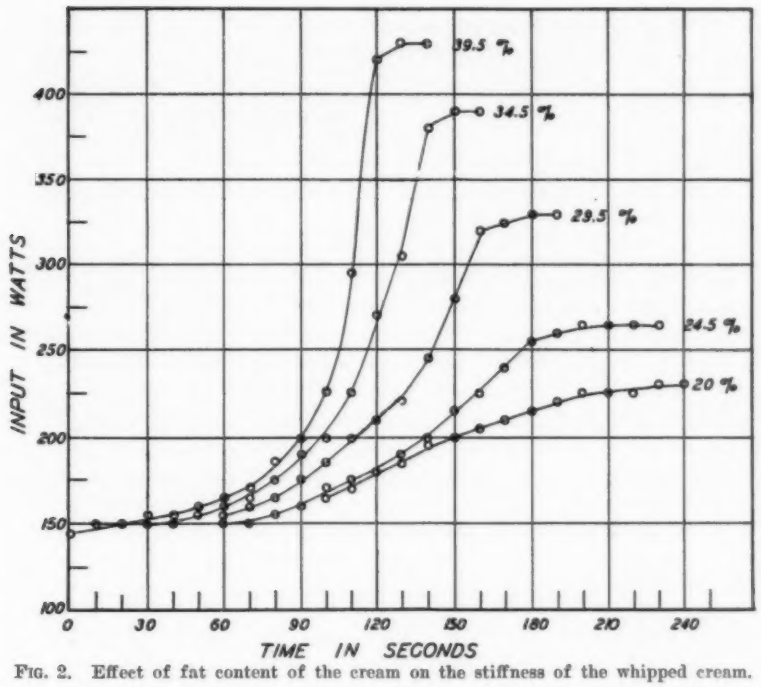


FIG. 2. Effect of fat content of the cream on the stiffness of the whipped cream.

APPLICATION OF METHOD

The wattmeter method for measuring the stiffness of whipping cream while the cream is being whipped should be useful in studies of cream whipping made either by the scientist or by the commercial dairyman. Measurements obtained by this method are certainly more accurate than visual observations and will serve several purposes. One use, for example, is partly illustrated in table 1, in which is indicated the effect of whipping a 33 per cent cream for varying periods of time on stiffness, overrun, amount of drainge, and percentage of butterfat in the drainage. It should be noted that at certain stages of the whipping procedure a variation of fifteen seconds in whipping time will materially affect the stiffness, the overrun, and the drainage of the whipped cream. A number of trials showed that the maximum overrun of the whipped cream occurs shortly before the maximum stiffness. It was found that the amount of drain decreased to a certain point with progressive whipping, reaching a minimum after the cream had been considerably over-whipped; but that upon still further whipping, it began to increase. The percentage of butterfat in the drain decreased with increased whipping until the cream was over-whipped, and

TABLE 1

Effect of whipping for varying periods of time on some of the properties of whipped cream

WHIPPING TIME		STIFFNESS	OVERRUN	DRAIN AFTER 24 HOURS	FAT IN DRAIN	REMARKS ON BODY OF WHIPPED CREAM
minutes	*seconds*	*watts*	*per cent*	*cc*	*per cent*	
Start		125				
1	30	147.5	71.8	(1)		Too soft
1	45	155	75.6	(1)		" "
2	0	165	79.1	(1)		" "
2	15	182.5	85.6	57	6.84	Sl. too soft
2	30	225.0	94.8	37	2.19	Good
2	45	265.0	94.8	23	1.34	"
3	0	262.5	85.1	20	1.23	Sl. soggy
3	15	250.0	74.7	19	1.38	Soggy
3	30	250.0	68.5	16	1.52	"
4	0	240.0	57.3	18	1.25	Very soggy
5	0	230.0	35.5	27	1.17	Buttery

Cream used in this experiment contained 33 per cent of butterfat and was aged for 48 hours at 38° F.

(1) Too soft to remain on 12 mesh-wire screening.

then remained fairly constant despite further whipping. It may be concluded, therefore, from the data in table 1 that it is very difficult to make accurate comparisons in cream whipping studies unless the consistency of the cream is accurately measured during the whipping process.

Accurate measurements of the consistency of cream during whipping are of practical value to the dairyman who sells whipping cream, because by the use of them he can set a standard for his cream and then check his cream each day in accordance therewith. If, then, the dairyman equips himself with a mechanical whipper and a wattmeter, he is able to produce a more uniform whipping cream or the finished product from day to day than he possibly could by unscientific means.

NUTRITIONAL ANEMIA, CALCIUM PHOSPHORUS AND NITROGEN BALANCE AND BONE COMPOSITION OF RATS FED RAW VERSUS PASTEURIZED MILK*

H. A. LASBY AND L. S. PALMER

Division of Agricultural Biochemistry, University of Minnesota, St. Paul.

PART I. NUTRITIONAL ANEMIA

Two groups of workers have concerned themselves with the relative values of sole diets of raw or pasteurized milk in producing nutritional anemia in the rat. Scott and Erf (1) report results showing that a special milk, when fed raw, allowed a better gain in weight and greater increase in red cell count and hemoglobin than when the same milk was fed after holding pasteurization. Some commercially pasteurized milk gave inferior results to those obtained with the special pasteurized milk. It is not stated whether the rats were fed individually or in groups and what their food consumptions were. Neither are any copper and iron analyses of the milk reported. Krauss, Erb, and Washburn (2) report results contrary to those of Scott and Erf. They fed milk from the same bulk to two groups of rats, one group receiving raw milk and the other the same milk after pasteurization. The animals in both groups developed nutritional anemia as characterized by a marked decrease in hemoglobin and erythrocyte count and the decreases were at the same rate in both groups. No data are given for the food intake or growth.

EXPERIMENTAL

Methods. The procedure of Elvehjem and Kemmerer (3) was followed in rendering the rats anemic. The young were weaned at three weeks, and then placed on the experimental diet of milk alone for an eight weeks period. The rats were housed in individual galvanized wire cages with $\frac{1}{2}$ inch mesh screen bottoms. The two series of experiments conducted were similar except in the milk fed. They were carried out by the paired feeding method using males only. Both members of any one pair were litter mates and as similar as possible as to weight at the beginning of the experimental period. Those on the sole milk diet received no water. The control group consisted of litter mates fed on the stock diet and water *ad lib.*

The rats in both series were weighed weekly and food consumption rec-

Received for publication November 6, 1934.

The data presented are taken in large part from the thesis of H. A. Lasby presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. All pertinent individual data are available for reference in the Division of Agricultural Biochemistry.

Paper No. 1320 Journal Series, Minnesota Agricultural Experiment Station.

ords were kept. Hemoglobin determinations were made at the time of weaning and weekly thereafter. The method used was that of Newcomer (4). Blood was obtained by cutting with a razor the tip of the tail, previously dipped in hot water to promote the flow of blood. Calcium chloride was first used to stop the flow of blood but later collodion was substituted, as it proved to be more satisfactory.

Series I. Seven pairs and three controls were used. The milk fed was obtained fresh daily, except Sunday, from the University of Minnesota Agricultural Experiment Station Dairy herd. The experiment was conducted during May, June and July when the cows were on pasture. No special containers were used for the collection of the milk. It was brought up from the barns in a tin pail from which it was transferred to glass containers for storage and pasteurization. A portion of each day's milk was fed raw to one group and a portion was pasteurized in glass and fed as such to the other. For pasteurization, the milk was placed in stoppered 1 l. Erlenmeyer flasks, which were then immersed to the neck in a water bath. A temperature of 62°–63° C. was maintained in the flask for 30 minutes. Subsequently the flasks and milk were cooled as rapidly as possible in running cold water and stored, as was the raw milk, in a cold room at 40° C. No attempt was made to standardize the fat content of the milk fed. Feedings were made morning and afternoon. Definite quantities of milk were measured by pipette into glazed porcelain ramekins placed on the screen bottom of the cage. Any milk unconsumed at the next feeding was measured.

Series II. Eleven pairs and three controls were started. The milk used was commercial[1] raw and pasteurized milk obtained during February and March, thus representing late winter feeding. Both were obtained from the same bulk. It contained 3.5 per cent butter fat. The pasteurization had been carried out in Yundt-Gridley stainless steel tanks by the holding process at 143.5° F. (62° C.) for 30 minutes. Quart aliquots were taken each day and delivered for consumption the following day, except Saturday, when two quarts of Friday's raw and pasteurized milk were delivered for Saturday's and Sunday's use.

Chemical analyses. Analyses for iron and copper were made on the raw and pasteurized milk. In series I random samples were taken every week and analyzed. In Series II, 100 cc. aliquots were taken daily and the analyses made on weekly composites. The method used for iron was that of Elvehjem and Hart (5), Kennedy (6), and Elvehjem (7). In Series I the copper was determined by the method of Elvehjem and Lindow (8)

[1] This milk was furnished through the courtesy of the Franklin Cooperative Creamery Association of Minneapolis. We acknowledge our indebtedness to them for it, and especially to Ass't. Supt. Walter Ahlstrom, for insuring the taking of samples and their delivery.

as modified by Gebhardt and Sommers (9) and in Series II by the method of Cherbuliez and Ansbacher (10) and Ansbacher, Remington and Culp (11). The latter was believed to be more accurate and certainly less tedious.

Results. Series I. The results obtained are presented graphically in figure 1. The mean hemoglobin value for the raw milk group decreased

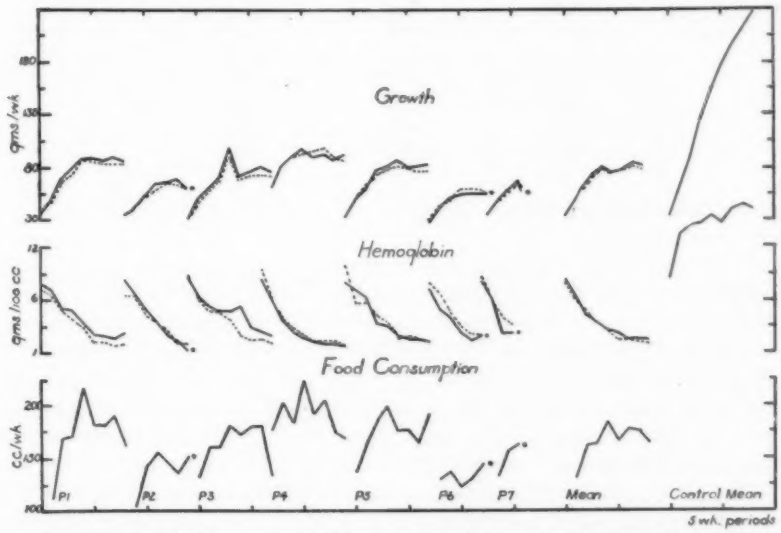


FIG. 1. Hemoglobin content of blood, body weight and milk consumption of individual rats fed raw and laboratory pasteurized summer milk from Station Dairy herd on pasture, together with mean values and the means of the blood hemoglobin and body weight of controls fed stock diet. The continuous lines represent raw milk feeding and the dotted lines pasteurized milk. The death of an animal before the end of the eight weeks period is starred.

from 7.80 to 2.31 gms. per 100 cc. while that of the pasteurized group decreased from 8.08 to 1.88 in eight weeks. The mean weight of the rats fed raw milk increased from 33.14 to 83.5 gms. in contrast to an increase from 33.71 to 78.5 gms. for those fed pasteurized milk. The data were treated statistically to test for the significance of the differences between the means of hemoglobin and the weight values obtained from the two groups of rats.

Using Fischer's application of 'Students'' distribution of t and the 5 per cent level of significance the data in table 1 show that the differences for each successive weekly interval were not significant except for the fifth week hemoglobin values. This cannot be considered important inasmuch

TABLE 1

Statistical significance of weekly differences in mean hemoglobin content of blood and body weight of rats fed raw and pasteurized milk in paired feeding tests

	CALCULATIONS FROM HEMOGLOBIN		CALCULATIONS FROM BODY WEIGHT	
Week	*t*	*P*	*t*	*P*
0	1.9277	0.0780	0.8738	0.4018
1	1.4279	.1786	0.8356	.4228
2	1.0463	.3194	0.3999	.6962
3	0.8717	.4020	1.5515	.1582
4	0.3036	.7704	0.5104	.6212
5	3.4662	.0062	0.4004	.6976
6	0.5587	.5914	0.1887	.8542
7	1.3859	.1086	2.0751	.0914
8	1.9765	.0966	1.7363	.1320

as no statistically significant difference occurred in either the preceding or succeeding week.

The copper and iron analyses of the raw and pasteurized milk gave results indicating that the same quantity of metals was present in each. Six iron analyses on raw milk made during the eight weeks period varied from 1.23 to 1.48 mgms. per liter with a mean of 1.35 and on the same milk after pasteurization from 1.20 to 1.48 mgms. per liter with a mean of 1.35. Six copper analyses made on the same milk varied from 0.11 to 0.17 mgms. per liter with a mean of 0.14 while those on the pasteurized milk varied from 0.12 to 0.16 mgms. per liter with a mean of 0.14 mgm.

Series II. Since the conditions used in laboratory pasteurization are not those under which market milk is treated, a series was run in order to determine whether commercial pasteurization had any effect on the severity of the anemia produced by feeding it as the sole diet to rats.

The results obtained are plotted in Figure 2. Pair number eight was omitted from all calculations because that member of the pair being fed pasteurized milk escaped from the cage one night and there was a subsequent rise in hemoglobin.

It may be seen that there was a much wider spread between the hemoglobin and growth curves of the two rats of a pair than in those of Series I. In all cases that member of a pair receiving pasteurized milk grew better and had more hemoglobin. If the rat on raw milk had a higher hemoglobin or weighed more at the beginning of the experiment, there was a subsequently poorer growth or a greater decrease in hemoglobins than on pasturized milk, resulting in lower values at the end of the experiment. In all cases the milk consumption was controlled by that member of the pair receiving raw milk.

Table 2 shows the statistical significance of the weekly differences of the mean values. It is seen that the differences in the mean hemoglobin values

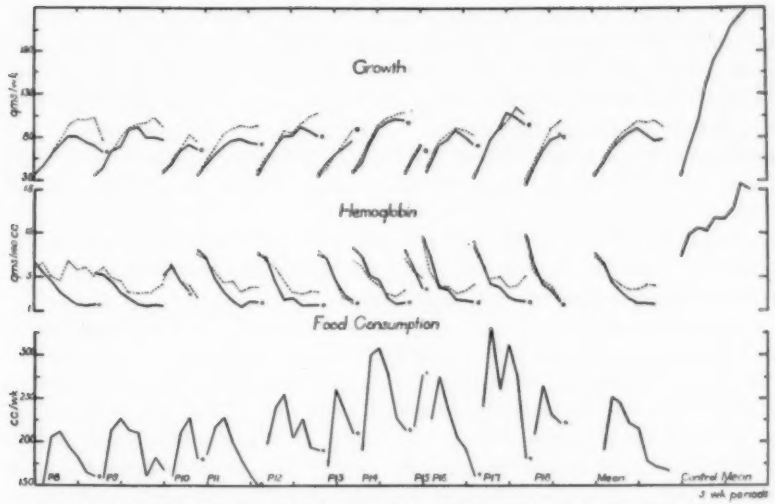


FIG. 2. Hemoglobin content of blood, body weight and milk consumption of individual rats fed commercially raw and pasteurized milk from winter feeding, together with mean values and the means of blood hemoglobin and body weight of controls fed stock diet. The continuous lines represent raw milk feeding, and the dotted lines pasteurized milk. The death of an animal before the end of the eight weeks period is starred.

were statistically significant at the end of the second week and thereafter to the end of the experiment. The fact that the differences of the first two weeks were highly insignificant does not mitigate against the significance found subsequently. A lapse of time before the appearance of the difference would be expected since the difference in the concentrations of copper and iron as shown by chemical analyses were small. When the mean weekly

TABLE 2

Statistical significance of weekly differences in mean hemoglobin content of blood and body weight of rats fed commercial raw and pasteurized milk in paired feeding tests

	CALCULATIONS FROM HEMOGLOBIN		CALCULATIONS FROM BODY WEIGHT	
Week	*t*	*P*	*t*	*P*
0	0.2822	0.7828	0.3419	0.8134
1	0.7104	.4866	0.9687	.3456
2	2.0342	.0576	0.6187	.5434
3	2.4231	.0284	2.0437	.0572
4	2.8503	.0130	1.0779	.2966
5	6.3123	.0000	1.2947	.2264
6	3.1061	.0112	0.5347	.6080
7	4.7452	.0090	3.7983	.0194

weights of the groups are considered, it may be seen that those fed pasteurized milk had consistently greater mean weights than those fed the raw milk. However, Table 2 shows that the difference was significant only in the third and seventh weeks. The significance in the third week was border line and since that in the seventh was based on only four individuals it is doubtful that it should be considered as truly significant. The greater differences observed in this series are no doubt due to the iron and copper intakes of the rats fed the pasteurized milk. However, it seems that although this greater intake was sufficient to produce significant differences in the hemoglobin values, slightly more would be necessary to produce equally significant differences in growth.

Chemical analyses of the commercial pasteurized milk showed that it differed in copper and iron content from the raw milk. Eight analyses for iron made during the eight weeks period showed for raw milk a range from 0.85 to 1.49 mgms. per liter with a mean of 1.13 while for pasteurized milk the range was from 1.06 to 1.64 mgms. per liter with a mean of 1.34. When the same milk was analyzed for copper, the raw milk showed a range from 0.15 to 0.24 mgms. per liter with a mean of 0.19 and the pasteurized milk ranged from a content of 0.23 to 0.32 mgms. per liter with a mean of 0.27. It seems evident that the milk picked up copper and iron in the milk plant although the stainless steel pasteurizing tanks could not have been responsible. It is this additional quantity of the metals in the pasteurized milk which is reflected in the higher hemoglobin and greater growth of the rats fed the commercially pasteurized milk.

As the experimental period progressed all rats in both series developed an appearance characteristic of nutritional anemia. In case of piebald rats the black fur became gray except in small area on the head. After the rats had been on the milk diet for some time it was noted that the incisor teeth were growing out without the characteristic brown pigment on the outer surfaces. In some the dark coloration was still visible on the distal ends of the teeth. This lack of tooth pigment seems to be a characteristic result of milk diets. Gross autopsies performed revealed that all organs and muscle tissue were bloodless in appearance. The caecum was almost invariably enlarged and often the heart. In Series I no difference could be noted in the appearance and behavior of those fed raw milk and those fed pasteurized milk. In Series II, however, there was a marked difference in the dispositions of the two groups. Those fed pasteurized milk were more active and eager for food, while the others tended to be inactive and huddled in the corners of the cages. In all cases the animals fed raw milk controlled the food consumption of the pair. The mortality in this series was much greater than in Series I. Of the eleven pairs started, only one survived the eight weeks experimental period. In the ten pairs in which early death occurred, nine of the ten rats dying were fed raw milk. Why the mortality

in this series was higher than in Series I is uncertain. However, it may have been due both to a smaller fat content if the commercial milk which was standardized to 3.5 per cent butter fat content, resulting in a lower caloric intake than in Series I, and to the fact that this milk which was secured for study in the latter part of the winter had a lower vitamin content, particularly vitamin A, as the result of the type of winter feeding practiced on the farms where the milk was produced.

PART II. CALCIUM, PHOSPHORUS, AND NITROGEN METABOLISM

That the heat treatment of milk alters certain of the milk salts is now definitely established. Whether this change alters their nutritive value is still unanswered. Considerable experimental nutritional work has been done on the comparison of the retention of the calcium, phosphorus, and nitrogen of raw and pasteurized milk. Most of the results are inconclusive and few can pass uncriticized. For an accurate comparison the raw and pasteurized milk fed must come from the same bulk and a sufficient number of animals must be used to insure the required degree of accuracy in the subsequent evaluation of the results. The effect of other articles of diet on the retention of these minerals is not known. There also appears to be species differences so that results obtained from one species are not necessarily applicable to another.

Daniels, Stuessy, and Francis (12) compared the growth of rats fed milk which had undergone various heat treatments. Ellis and Mitchell (13) carried out carefully controlled calcium balance studies with rats fed raw or pasteurized milk plus a basal ration containing a small amount of calcium. They found that the animals fed raw milk gained more in weight and showed a higher calcium content of the carcase than those fed pasteurized milk. In both cases the results were statistically significant. However, when the average calcium retention was as high as 98 per cent for rats on raw milk and 92 per cent for rats on pasteurized milk the calcium intake was probably too low to be of any practical importance.

Krauss, Erb, and Washburn (2) measured the ability of rats to use calcium and phosphorus from raw pasteurized milk by analyzing the femurs of rats after a period of feeding on the milk as a sole diet. No differences in the percentage of ash, calcium and phosphorus were found.

EXPERIMENTAL

Methods. Male rats 10 weeks of age, and weighing from 225 to 292 gms. were selected from an inbred strain selected for high efficiency of food utilization during growth. At this age the rats selected were still storing calcium, their normal growth curve was still a straight line, and their efficiency was known. The paired feeding method was used. Male litter mates were paired as nearly as possible for weight. The study included

six pairs from two litters. One member of each pair was fed raw milk and the other pasteurized milk from the same bulk. The milk fed was from the University of Minnesota Agricultural Experiment Station herd. The experiment was conducted in late September and early October. The method of pasteurizing was the same as that used in Series I of the anemia studies. Seven day collection periods were used. The rat was always placed on the particular diet to be used three days before collections were begun. Three successive collection periods were used for each pair. The order of feeding for one member of a pair was raw, pasteurized, and raw, and for the other pasteurized, raw, and pasteurized. This alternation was used in order to minimize the possible influence of the order of feeding the two milks. The milk was fed twice a day, morning and late afternoon. To the morning feeding there was added 1 cc. of a slightly acidulated solution of ferric chloride and copper sulfate made up so that 1 cc. contained 0.4 mgms. of Fe and 0.32 mgms. of Cu in order to prevent nutritional anemia. The milk thus supplemented was completely consumed by both members of a pair before more was fed. The milk was measured out by a pipette and any unconsumed after 24 hours was measured.

The cages used were especially designed for metabolism studies. The rats were weighed weekly and food consumption records kept. The urine and feces were collected after each period and analyzed for calcium, phosphorus, and nitrogen.

Similar analyses were made on aliquots of weekly composite samples of the raw and pasteurized milk fed. The milk was preserved with formaldehyde until the analyses were made. Fifteen pairs of balance determinations were made using six pairs of rats. By subtracting the total amount of a mineral excreted in the urine and feces from the intake as supplied by the milk, the balance was obtained. For the sake of comparison, the balance values were then calculated on the basis of the percentage of the intake retained. In order to compare the percentages of the intake of the respective minerals which were retained on the diets of raw and pasteurized milk, the mean values were calculated.

Chemical analyses. The calcium and phosphorus determinations were made on the samples by the method of Morris, Nelson, and Palmer (14). The nitrogen was determined by semi-micro Kjeldahl method of Cavett (15).

Results. The results obtained are presented in Table 3. The significance of the difference was then determined as in Part I.

The mean percentages of intake retained together with the corresponding probabilities are given in Table 4.

There was no significant difference between the retentions on the diets of raw and pasteurized milk. The slight differences which were found favored the pasteurized milk.

TABLE 3

Calcium, phosphorus and nitrogen balances

PAIR NO.	RAT NO.	DIET	Ca INTAKE	TOTAL Ca EXCRETION	Ca BALANCE	Ca INTAKE RETAINED	P INTAKE	TOTAL P EXCRETION	P BALANCE	P INTAKE RETAINED	N INTAKE	TOTAL N EXCRETION	N BALANCE	N INTAKE RETAINED
			mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1.	1728	1. raw	539.8	344.4	195.4	63.8	435.7	250.9	184.8	57.6	2633.8	2141.8	492.0	81.3
	1730	1. past.	531.7	394.4	137.3	74.2	423.4	267.6	155.8	63.2	2553.6	1814.6	737.2	71.1
2.	1729	1. raw	383.2	262.8	120.4	68.6	302.9	212.1	90.8	70.1	1995.5	1531.9	463.6	78.1
		2. past.	631.8	478.5	153.3	75.7	477.9	287.2	190.7	60.1	3068.0	2470.2	597.8	80.5
	1731	1. past.	371.0	239.2	131.8	64.5	291.3	192.6	98.7	66.1	1807.5	1519.1	288.4	84.1
		2. raw	625.6	478.7	146.9	76.5	486.2	280.3	205.9	57.7	2984.8	2268.7	716.1	76.0
3.	1733	1. raw	532.7	300.1	232.6	56.4	429.9	273.2	156.7	63.6	2598.8	2756.1	842.7	67.6
		2. past.	637.9	378.8	259.1	59.4	486.2	221.5	261.0	45.9	3097.5	1960.9	1136.6	63.3
		3. raw	609.7	450.1	159.6	73.8	465.9	270.4	195.5	58.1	2930.1	2255.0	675.1	77.0
	1735	1. past.	537.7	297.1	140.6	73.9	428.1	257.7	170.4	60.2	2582.1	1833.2	748.9	71.0
		2. raw	631.6	354.0	267.6	57.6	490.9	234.4	256.5	47.8	3013.5	2358.8	654.7	78.3
		3. past.	620.1	385.0	235.1	62.1	458.5	244.6	213.9	53.4	2985.3	2414.3	571.0	80.9
4.	1766	1. raw	625.0	393.8	231.2	60.3	413.9	239.8	174.1	57.9	2391.4	1852.1	539.3	77.5
		2. past.	604.5	487.9	116.6	80.7	464.7	276.1	188.6	59.4				
		3. raw	603.8	490.4	113.4	81.2	474.6	264.7	209.9	55.8				
	1767″	1. past.	576.3	267.6	308.7	46.4	298.7	241.5	157.2	60.6	2376.0	1725.0	651.0	72.6
		2. raw	624.4	436.5	187.9	69.9	445.6	252.9	192.7	56.8				
		3. past.	622.1	453.1	169.0	72.7	457.0	254.5	202.5	55.7				
5.	1765	1. raw	580.8	333.4	247.4	57.4	384.6	224.7	159.9	58.4	2222.2	1775.9	446.3	79.9
		2. past.	612.3	422.2	190.1	69.0	470.7	267.8	202.9	56.9	2593.5	2235.1	358.4	86.2
		3. raw	639.8	479.4	160.4	74.9	502.9	286.1	216.8	56.9	2756.7	2304.9	451.8	83.6
	1764″	1. past.	561.6	318.9	242.7	56.8	388.6	191.2	197.4	75.0	2231.3	1673.7	557.6	75.0
		2. raw	628.4	483.0	145.4	76.9	448.4	286.0	162.4	81.3	2650.5	2150.9	499.6	81.3
		3. past.	643.1	466.4	176.7	72.5	472.4	298.2	174.2	83.0	2787.6	2313.3	474.3	83.0
6.	1767′	1. raw	498.0	335.2	162.8	67.4	329.8	211.5	118.3	73.0	1905.5	2391.0	514.5	73.0
		2. past.	483.4	346.1	137.3	71.6	371.6	261.4	110.2	69.8	2047.5	1429.8	617.7	69.8
		3. raw	538.5	491.2	147.3	72.7	423.3	240.6	182.7	75.5	2320.2	1751.1	569.1	75.5
	1769	1. past.	382.8	267.9	114.9	70.0	264.9	214.7	50.2	89.8	1578.5	1417.8	160.7	89.8
		2. raw	480.2	352.2	128.0	73.3	342.7	225.6	117.1	58.8	2025.4	1190.9	834.5	58.8
		3. past.	546.6	409.4	137.2	74.9	401.5	252.3	149.2	80.7	2369.4	1912.9	256.5	80.7

BONE ANALYSES

Methods. For the purpose of determining whether the calcium and phosphorus of raw and pasteurized milk were equally available for bone formation, analyses were made on bones of the rats used in Series II of the anemia studies. The femurs and tibias were removed immediately after death, stripped of adhering flesh, and stored until the analyses were made.

TABLE 4

Mean percentage retention of calcium, phosphorus and nitrogen from raw and pasteurized milk and P value of differences

ELEMENT	RETENTION ON RAW MILK	RETENTION ON PASTEURIZED MILK	P
	per cent	*per cent*	
Calcium	68.9	68.3	0.6009
Phosphorus	58.5	60.5	0.1120
Nitrogen	76.0	77.5	0.1616

At that time the femur and tibia of one leg from each animal were selected, crushed in filter paper, and extracted with alcohol for 48 hours and with ether for 24 hours. The percentage of ash in the bones was determined and the ash analyzed for calcium and phosphorus according to the method of Morris, Nelson, and Palmer (14). Weekly aliquots of the milk were also analyzed for calcium and phosphorus by the same method. The mean calcium values of the weekly aliquots of raw and pasteurized milk were 11.96 mgm. and 12.01 mgm. per liter respectively. The corresponding phosphorus values were 8.92 mgm. and 8.96 mgm. per litter of raw and pasteurized milk.

Results. Results of the bone analyses of the eleven pairs of rats are presented in Table 5.

These data were treated statistically by the method previously referred to. A probability of 0.054 was obtained that the percentage of ash in the bones of rats fed raw milk was significantly different from that of the rats fed pasteurized milk. This is a border line value. The percentage of ash was then correlated with the age of the rat, since the age might influence the percentage of ash and the rats used were of different ages depending on the time during the experimental period at which death occurred. A correlation coefficient equal to + 0.7638 was obtained but this was not significant for the number of samples correlated. Since the femur and tibia of the other hind leg of the rats were also available, the length of these was obtained by means of calipers. The means of these data also are shown in Table 5. The total length of the two bones was then correlated with the percentage of ash. This was done because the rats fed pasteurized milk grew better but their bones showed a lower ash content than did the bones

TABLE 5

Calcium and phosphorus content and length dry-extracted femur plus tibia of rats dying from anemia after consuming commercial raw and pasteurized milk

MILK	BONE COMPOSITION			TOTAL LENGTH FEMUR PLUS TIBIA
	Total Ash	Ca	P	
	per cent	*per cent*	*per cent*	mm.
Raw	58.93	22.13	9.38	5.326
Pasteurized	56.99	21.67	9.16	5.389

of the rats fed raw milk. Hence, if a longer bone showed a lower ash content, it should contain more organic and less mineral matter. The correlation coefficient between the length of the leg bones and the ash content in the raw group was +0.6238 and in the pasteurized group +0.2493, neither of which are significant. It seems, therefore that the difference in the mean percentage of ash in the bones of the two groups is probably not significant.

SUMMARY AND CONCLUSIONS

Experiments designed to compare the severity of nutritional anemia developed in comparative groups of rats when one group was fed raw milk and the other the same milk after pasteurization have been carried out. The results of the experiment show:

1. When the pasteurization of milk is carried out in glass in the laboratory, the severity of the anemia and the growth of the rats are not significantly different on diets of raw or pasteurized milk.

2. The iron and copper contents of milk are not affected by pasteurization in glass in the laboratory.

3. When the pasteurization of milk is carried out commercially, the anemia developed may be less severe than on raw milk and the growth better.

4. The iron and copper contents of milk commercially pasteurized may be greater than that of the milk before the heat treatment due to contact with the metallic equipment in use in commercial milk plants.

Experiments designed to compare the calcium, phosphorus, and nitrogen balance metabolism of paired rats, one of which received a sole diet of raw milk and the other a sole diet of the same milk after holding pasteurization in glass in the laboratory, have been carried out. The results of these experiments lead to the following conclusions:

1. The calcium retentions are the same on the two diets.

2. The phosphorus retentions are slightly but not significantly greater on pasteurized milk.

3. The nitrogen retentions are slightly but not significantly greater on pasteurized milk.

Analyses of the percentage of ash, calcium and phosphorus have been made on dry-extracted femur-tibia bones of rats fed a sole diet of raw or commercially pasteurized milk.

The following results were found:

1. The bones of the rats fed raw milk had a higher percentage of ash than those of the rats fed pasteurized milk. The difference was probably not significant.

2. The mean calcium and phosphorus contents of the bones of the rats fed raw milk were slightly but not significantly higher than those of the rats fed pasteurized milk. The ash, calcium, and phosphorus differences while not significant may have been due, in part, to the better growth of the rats fed the pasteurized milk.

3. The mean calcium and phosphorus contents of the milk before and after pasteurization were the same.

REFERENCES

(1) Scott, E., and Erf, L. A. Ohio tests prove natural milk is best. Jersey Bull. **50**: 210 (1931).

(2) Krauss, W. E., Erb, J. H., and Washburn, R. G. The effect of pasteurization on some of the nutritive properties of milk. Ohio Agr. Exp. Sta. Bull. **518** (1933).

(3) Elvehjem, C. A., and Kemmerer, A. R. An improved technique for the production of nutritional anemia. J. Biol. Chem. **93**: 189–195 (1931).

(4) Newcomer, H. S. Absorption spectra of acid hematin, oxyhemoglobin, and carbon monoxide hemoglobin. A new hemoglobinometer. J. Biol. Chem. **37**: 465–496 (1919).

(5) Elvehjem, C. A., and Hart, E. B. Quantitative methods for the determination of iron in biological materials. J. Biol. Chem. **67**: 43–51 (1926).

(6) Kennedy, R. P. The quantitative determination of iron in tissues. J. Biol. Chem. **74**: 385–391 (1927).

(7) Elvehjem, C. A. A note on the determination of iron in milk and other biological materials. J. Biol. Chem. **86**: 463–467 (1930).

(8) Elvehjem, C. A., and Lindlow, C. W. The determination of copper in biological materials. J. Biol. Chem. **81**: 435–443 (1929).

(9) Gebhardt, H. T., and Sommers, H. H. Determination of copper in dairy products. J. Ind. Eng. Chem. Anal. Ed. **3**: 24–26 (1931).

(10) Cherbuliez, E., and Ansbacher, S. Sur le dosage du cuivre dans des matieres organiques. Helv. Chim. Acta **13**: 187–194 (1930).

(11) Ansbacher, S., Remington, R. E., and Culp, F. B. Copper determination in organic matter. J. Ind. Eng. Chem. Anal. Ed. **3**: 314–317 (1931).

(12) Daniels, A. L., Stuessy, S., and Francis, E. The nutritive value of boiled milk. Am. J. Dis. Children **11**: 45–54 (1916).

(13) Ellis, M., and Mitchell, H. H. Effect of pasteurization of milk on the utilization of its calcium for growth in the rat. Am. J. Physiol. **104**: 1–9 (1933).

(14) Morris, H. P., Nelson, J. W., and Palmer, L. S. Quantitative determination of calcium, magnesium, and phosphorus in feedstuffs and cattle excreta. J. Ind. Eng. Chem. Anal. Ed. **3**: 164–167 (1931).

(15) Cavett, J. W. An improved micro Kjeldahl method. J. Lab. Clin. Med. **17**: 79–82 (1931).

American Dairy Science Association Announcements

ANNUAL MEETING
AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY FARM, ST. PAUL

June 24 to 27, 1935

GENERAL INFORMATION

There has been a slight change in the general plan of the meeting from that announced in the February issue of this Journal. It is now planned to have the opening session Monday evening, June 24th, at which there will be the usual addresses of welcome and opportunity to transact such matters of business as the officers desire to present to the Association. A second and concluding business session will be held Wednesday afternoon, June 26th. The opening session will be followed by a general get-together in the Dormitory Parlors where it is expected that most of the members will have secured rooms.

Plans are taking shape for the opening scientific session, Tuesday morning, June 25th. This is to be a symposium entitled "Improving the Germ Plasm of Domestic Plants and Animals" which will be held jointly under the auspices of Section O, A. A. A. S., the American Dairy Science Association, the Corn Belt Section of the American Society of Agronomy, the American Society of Horticultural Science Great Plains Section, the American Society of Plant Physiologists, and possibly also the American Phytopathological Society. Our own Association will be represented on this symposium by Dr. Jay L. Lush, of Iowa State College. The program committee hopes that this unusual opportunity to meet with our colleagues and friends in other fields of agricultural science will prove to be the special feature of the 1935 meeting which will long remain a pleasant memory as well as be an inspiration to all of us.

SECOND CALL FOR PAPERS AND ABSTRACTS

Members are again invited to send titles of papers for the Sectional Scientific sessions. Each title should be accompanied by an abstract of 300 words or less. *All communications relative to papers should be sent directly to the chairman of the Program Committee.* All titles and abstracts must be received by May 1st in order that the program may be arranged and printed in the June issue of this Journal and the abstracts be made available in printed form for the meeting. Authors are asked to indicate the

section before which they wish to appear. Authors who submit titles and abstracts and later learn that they will not be able to attend the meeting are urged to advise the chairman if possible by May 10th in order that the paper may be placed at the end of the program for presentation by vote of the membership present.

L. S. PALMER, *Chairman*
Program Committee
University Farm, St. Paul

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF
AMERICAN DAIRY SCIENCE ASSOCIATION

A. C. DAHLBERG, Editor
Geneva, N. Y.

Associate Editors

L. S. PALMER
St. Paul, Minn.

C. W. LARSON
Buffalo, N. Y.

H. A. RUEHE
Urbana, Ill.

E. G. HASTINGS
Madison, Wis.

E. B. MEIGS
Beltsville, Md.

PAUL F. SHARP
Ithaca, N. Y.

O. F. HUNZIKER
Chicago, Ill.

L. A. ROGERS
Washington, D. C.

C. E. GRAY
San Francisco, Cal.

Committee on Journal Management

O. F. HUNZIKER, Chairman
Chicago, Ill.

R. R. GRAVES
Washington, D. C.

A. A. BORLAND
State College, Pa.

The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to A. C. Dahlberg, New York Agricultural Experiment Station, Geneva, New York. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

Subscriptions. Price; $5.00 per volume in United States, Canada and countries within the postal union; $5.50 in countries outside the postal union. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume unless otherwise stated. Renewals should be made promptly to avoid a break in the series.

Subscriptions and Advertising should be mailed direct to the Science Press Printing Company, Lime and Green Streets, Lancaster, Pennsylvania.

All subscriptions from the British Isles and British Empire (with the exception of Canada) should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Covent Garden, London, W.C.2, England.

Correspondence regarding business policies of the Journal should be addressed to the Editor. The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Chr. Hansen's Laboratory in Milwaukee

USING THE MOST MODERN METHODS

It is of utmost importance to the dairy industry that it have preparations of dependable quality and known uniformity. To provide such assurance, we operate this completely modern laboratory where Hansen's Rennet, Cheese Color, Butter Color and Lactic Ferment Cultures are prepared under constant scientific control.

CHR. HANSEN'S LABORATORY, INC.
Milwaukee, Wis.

Facts you should know about

GLASS-LINED PASTEURIZERS

FACT II 4

*IV. ***Rate of Heat Transfer***—In the rapidity with which a pasteurizer heats and cools and the evenness with which temperature is held lies an important cost to the dairyman and a determining factor in quality.

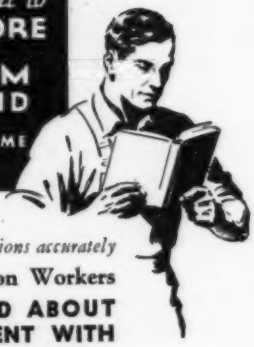
Repeated competitive tests have shown that Pfaudler Pasteurizers heat faster, hold most evenly, cool rapidly. Such performance is due to correct insulation plus heating with ***steam*** through glass lined steel.

This is most important. It simplifies the heating problem, and eliminates costly temperature controls. This reduces the initial cost of the equipment. It increases speed of heating and simplifies operating practice. The glass lining serves as an automatic check, preventing scorching. Of course this advantage is possible only with glass lined steel.

THE PFAUDLER CO.
1515 Gas & Electric Bldg., Rochester, N. Y.

PFAUDLER

Farmers want to **KNOW MORE** *about* **CALCIUM CYANAMID** *the* NITROGEN + LIME FERTILIZER



To answer their questions accurately
Experiment Station Workers
SHOULD READ ABOUT AND EXPERIMENT WITH
'AERO' CYANAMID
as a fertilizer for haylands and pastures.

A bag of AERO Cyanamid will gladly be supplied, free of charge, to any reader of this Journal who desires to give the material a field trial.

Write **AMERICAN CYANAMID COMPANY**
30 ROCKEFELLER PLAZA NEW YORK

Manufacturers of
'AERO' CYANAMID – 'AMMO-PHOS'

SUPPLEMENT TO

JOURNAL OF DAIRY SCIENCE

Vol. XVIII March, 1935 No. 3

Corrections for article by

H. A. LASBY and L. S. PALMER

Entered as second-class mail matter April 13, 1934, at the post office at Lancaster, Pa.
Under the act of March 3, 1879

NUTRITIONAL ANEMIA, CALCIUM PHOSPHORUS AND NITROGEN BALANCE AND BONE COMPOSITION OF RATS FED RAW VERSUS PASTEURIZED MILK

H. A. Lasby and L. S. Palmer

Due to a number of unfortunate errors in the calculation of the chemical data and statistical significance of the results the following corrections are required in the paper bearing the above title in this Journal Vol. *18*, No. 3, March 1935, pages 181 to 192.

On page 183 last paragraph ending on page 184 read the following in place of the entire paragraph: Using 'Student's' formula for t as applied to small samples, calculating the probability (P) as (1-a) from 'Student's' tables[1] for $\frac{1}{2}$ (1 + a) and selecting a 5 per cent level for significance the data in Table 1 show that the differences for each successive weekly interval are not significant.

On page 185, line 1 read "third week" in place of "second week."

On page 186, lines 3, 4, 5 and 6 read the following: However, Table 2 shows that the difference was significant only in the third week, and, being only slightly significant as well as being followed by differences which were not significant, cannot be given much consideration.

On page 188 omit the last sentence beginning, "The slight differences—."

On page 190, lines 16 and 17 read the following in place of the entire sentence: *Results.* Results of bone analyses of ten of the eleven pairs of rats are presented in Table 5, the data from pair No. 15 being omitted from the calculations because the animals remained in the experiment only 17 days.

On page 190, line 19, read 0.0076 for 0.054; line 21, read "significant" for "border line"; line 25, read +0.6259 for +0.7638; lines 24, 25 and 26 read in place of the sentence beginning, "A correlation—" the following: A correlation coefficient of +0.6259 was obtained for the bone ash of the rats fed the raw milk and a correlation coefficient of +0.4043 for the bone ash of the rats fed pasteurized milk, the value for the raw milk fed animals having a border line significance for the number of samples.

On page 191, line 4, read +0.5088 for +0.6238 and read +0.3690 for +0.2493; line 6, read "statistically" for "probably not." Add the following: The biological significance of the result is rendered improbable by the fact that similar calculations for the calcium and phosphorus content of the same bones showed probability values for significance of 0.3492 and 0.3839, respectively. Line 28, read: 1. The calcium retentions were slightly greater on the raw milk but the difference is not statistically significant; line 29, read: 2. The phos-

[1] Metron 5:26-29. 1925.

phorus retentions were the same on the two diets; line 32 read "raw" for "pasteurized."

On page 192, lines 6 and 7, read in place of the sentence beginning. "The difference—," the following: The difference is statistically but not biologically significant and is not explained on the basis of the better growth of the rats receiving the pasteurized milk; lines 10, 11 and 12, omit the sentence beginning "The ash—."

In addition to the above corrections substitute the following tables, 1, 2, 3, 4 and 5, for the corresponding tables on pages 184, 185, 189, 190 and 191, respectively.

TABLE 1

	CALCULATIONS FROM HEMOGLOBIN		CALCULATIONS FROM BODY WEIGHT	
Week	*t*	*P*	*t*	*P*
0	0.5619	0.5850	0.2552	0.8042
1	0.1996	.8452	0.2443	.8114
2	0.2012	.8358	0.1218	.9052
3	0.2923	.7752	0.4542	.6582
4	0.1075	.9166	0.1649	.8724
5	1.1926	.2608	0.1848	.8572
6	0.2547	.8056	0.0498	.9614
7	0.9271	.3902	0.8960	.4048
8	1.3656	.2214	0.7519	.4812

TABLE 2

	CALCULATIONS FROM HEMOGLOBIN		CALCULATIONS FROM BODY WEIGHT	
Week	*t*	*P*	*t*	*P*
0	0.1124	0.9118	0.3153	0.7564
1	0.4161	.8624	0.8945	.3830
2	1.8814	.0920	0.6890	.4998
3	2.2815	.0366	2.2127	.0402
4	2.8422	.0120	1.0197	.3234
5	5.5720	.0002	1.1952	.2596
6	6.5607	.0000	2.0302	.0700
7	3.6187	.0154	2.0256	.0894

TABLE 3

PAIR NO.	RAT	DIET	Ca INTAKE	TOTAL Ca EXCRETION	Ca BALANCE	Ca INTAKE RETAINED	P INTAKE	TOTAL P EXCRETION	P BALANCE	P INTAKE RETAINED	N INTAKE	TOTAL N EXCRETION	N BALANCE	N INTAKE RETAINED
			mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1.	1728	1. raw	556.3	344.6	211.7	38.06	439.0	250.9	188.1	42.84	2887.2	2146.1	741.1	25.67
	1730	1. past.	447.1	394.6	52.5	11.74	413.1	267.7	145.4	35.20	2562.6	1821.1	741.5	28.93
2.	1729	1. raw	383.8	263.3	120.5	31.39	302.9	212.1	90.8	29.97	1992.2	1536.5	455.7	22.87
		2. past.	624.5	489.0	153.5	21.71	503.4	287.2	216.2	42.95	2953.6	2470.6	483.0	16.35
	1731	1. past.	315.4	245.3	70.1	22.20	291.3	192.4	98.9	33.96	1807.5	1389.7	417.8	23.12
		2. raw	632.8	478.8	154.0	24.35	520.0	280.3	239.7	46.10	2901.6	2268.7	632.9	21.81
3.	1733	1. raw	525.6	300.2	225.4	42.88	414.7	273.2	141.5	34.13	2727.9	1754.5	973.4	35.68
		2. past.	645.2	379.1	266.1	41.25	456.7	223.0	233.7	51.17	3213.0	1960.9	1252.1	38.97
		3. raw	610.3	472.5	137.8	22.58	464.8	270.5	194.3	41.80	2930.1	2420.0	510.1	17.41
	1735	1. past.	452.1	297.1	155.0	34.28	417.7	256.4	161.3	38.61	2591.2	1833.0	758.2	29.26
		2. raw	625.8	364.1	261.7	41.82	456.7	234.4	222.3	48.67	3108.0	2360.6	747.4	24.05
		3. past.	620.1	397.9	222.2	35.83	458.5	244.9	213.6	46.59	2985.3	2415.1	570.2	19.10
4.	1766	1. raw	625.5	393.9	231.6	37.02	413.9	239.8	174.1	42.06	2391.5	1849.6	541.9	22.66
		2. past.	604.5	488.0	116.5	19.27	465.2	276.1	189.1	40.66				
		3. raw	603.8	490.4	113.4	18.77	474.6	264.8	209.8	44.20				
	1767"	1. past.	576.3	367.5	308.7	36.23	398.7	241.4	157.3	39.46	2367.4	1723.5	643.9	27.20
		2. raw	624.5	436.3	188.2	30.13	442.7	253.0	189.7	42.86				
		3. past.	622.6	448.3	174.3	28.00	476.6	254.7	221.9	46.56				
5.	1765	1. raw	581.2	333.4	247.8	42.64	384.6	224.7	159.9	41.58	2222.2	1769.7	452.5	20.36
		2. past.	612.3	421.9	190.4	31.09	471.2	269.5	201.7	42.81	2593.5	2264.2	329.3	12.70
		3. raw	639.9	479.5	160.4	25.07	502.9	284.2	218.7	43.49	2756.8	2307.0	449.8	16.32
	1764"	1. past.	561.6	319.5	242.1	43.11	388.6	191.4	197.2	50.76	2307.1	1672.5	634.6	27.51
		2. raw	628.4	464.7	163.7	26.06	445.6	277.0	168.6	37.83	2650.5	2153.9	496.6	18.74
		3. past.	643.5	461.1	182.4	28.34	492.7	294.7	198.0	40.18	2787.6	2309.3	478.3	17.16
6.	1767'	1. raw	498.4	337.0	161.4	32.39	329.8	212.0	117.8	35.71	1905.5	1388.9	516.6	27.11
		2. past.	483.4	346.3	137.1	28.36	372.0	229.6	142.4	38.28	2047.5	1430.7	616.8	30.12
		3. raw	538.5	391.2	147.3	27.36	423.3	270.6	182.7	43.16	2320.2	1753.8	566.4	24.41
	1769	1. past.	382.9	280.3	102.6	26.80	264.9	219.4	45.5	17.16	1572.8	1314.4	258.4	16.43
		2. raw	480.2	352.2	128.0	26.66	340.5	225.6	114.9	33.74	2025.5	1189.5	836.0	41.27
		3. past.	547.0	409.4	137.6	25.15	418.8	252.2	166.6	39.77	2369.5	1913.0	456.5	19.26

TABLE 4

ELEMENT	RETENTION ON RAW MILK	RETENTION ON PASTEURIZED MILK	P
	per cent	*per cent*	
Calcium	31.15	28.89	0.4608
Phosphorus	40.54	40.27	.9138
Nitrogen	24.49	23.55	.7506

TABLE 5

MILK	BONE COMPOSITION			TOTAL LENGTH FEMUR PLUS TIB[1]A
	Total Ash	Ca	P	
	per cent	*per cent*	*per cent*	*mm.*
Raw	59.29	21.97	9.36	5.353
Pasteurized	56.56	21.53	9.12	5.536

DIFCO

The Detection of Coli in Milk

Bacto-Brilliant Green Bile 2%

Bacto-Brilliant Green Bile 2% is an excellent medium for the detection of coli in milk and other dairy products. The medium is readily prepared for use and requires neither adjustment of its reaction nor filtration of the solution. Results obtained by the direct inoculation of milk into fermentation tubes of this medium are reliable and comparable.

Bacto-Gentian Violet Lactose Bile

Bacto-Gentian Violet Lactose Bile is prepared according to the formula of Kessler and Swenarton. The production of gas following the inoculation of contaminated milk samples into tubes of this medium is readily observed, and is an indication of the presence of coli. According to Kessler and Swenarton such a positive test requires no further confirmation.

Specify "Difco"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT, MICHIGAN

THE SCIENCE PRESS
PRINTING COMPANY

Printers of
Scientific and Educational
Journals, Monographs and
Books

LIME AND GREEN STREETS
LANCASTER, PA.

Your advertisement is being read in every State and in 32 Foreign Countries

More EFFICIENT Sterilization

You can *profit* from the very definite advantages of Wyandotte Steri-Chlor. Check over these advantages and you'll want to try this more efficient germicide and deodorant.

1/ Makes a clear solution
2/ Does not deteriorate while dry in the container
3/ Has a low alkalinity
4/ Holds its efficiency remarkably long while in solution
5/ Doesn't lose chlorine strength in hot solution
6/ Is a remarkable de-odorizer
7/ Has no undesirable effects on equipment, containers or contents

You will find it *profitable* to use Wyandotte Steri-Chlor for germicidal action and deodorizing. Your Wyandotte Service Man will give you full details or

Ask your Supply Man

WYANDOTTE STERI-CHLOR

Wyandotte

The J. B. Ford Company — Wyandotte, Michigan

THE RIGHT MICROSCOPE

With which to

EXAMINE

Filter Pads

IN testing for extraneous matter in butter and cream this Spencer No. 58 Wide-Field Binocular Microscope is ideal for the examination of the filter pad through which the sample has been drawn. It has an instantly variable magnification of 8ix, 43x, and 83x. Rat hair can be easily identified.

The specific advantages of this Spencer No. 58 Microscope are:

(1) The image is NOT inverted nor reversed.
(2) The image is stereoscopic and third dimensional.
(3) Freedom from eyestrain.
(4) Wide Field.

Write us for complete information and description. Address Dept. W-S.

Spencer Lens Company

BUFFALO — NEW YORK

Your advertisement is below result in every State and in 35 Foreign Countries.